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Kalns et al.

(54) METHODS AND COMPOSITIONS FOR BIOMARKERS OF FATIGUE

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- (60) Provisional application No. 61/559,632, filed on Nov. 14, 2011.
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2800/306 (2013.01)

(58) Field of Classification Search

None

See application file for complete search history.

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(57) ABSTRACT

The present invention provides methods and compositions for identifying fatigue, disease states associated with fatigue, recovery from fatigue and/or physical performance capability in a subject.

1 Claim, 6 Drawing Sheets

FIG. 1

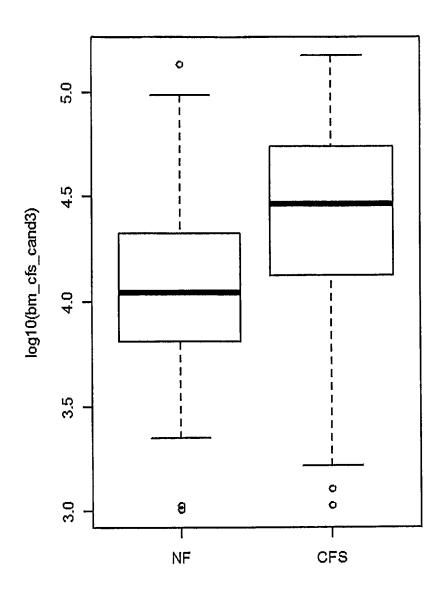


FIG. 2

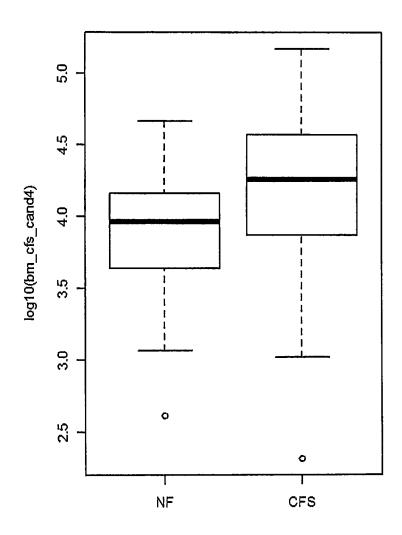


FIG. 3

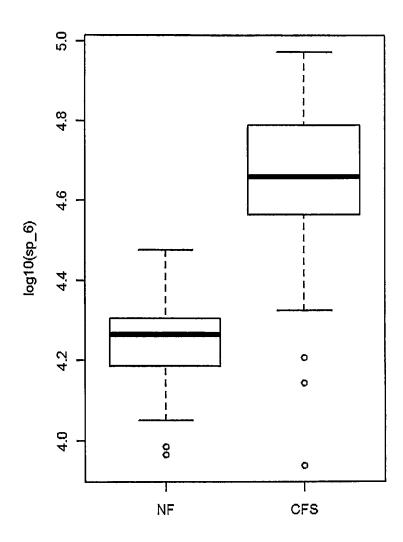


FIG. 4

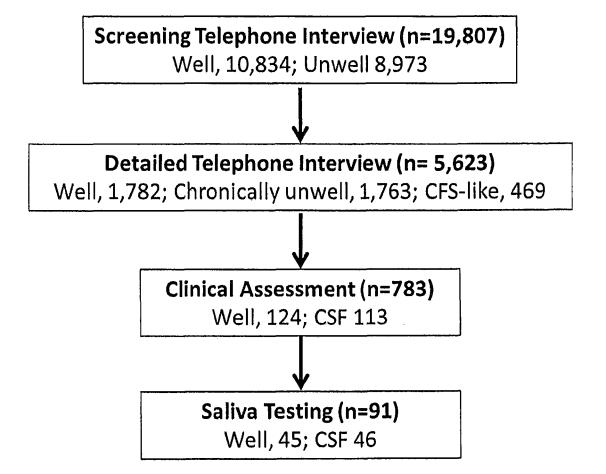


FIG. 5

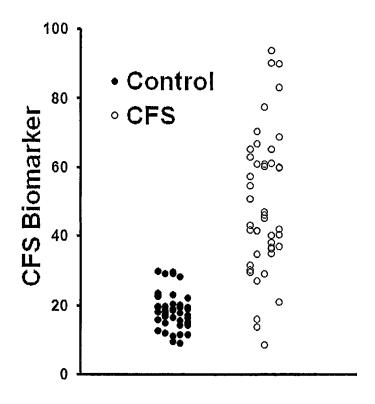
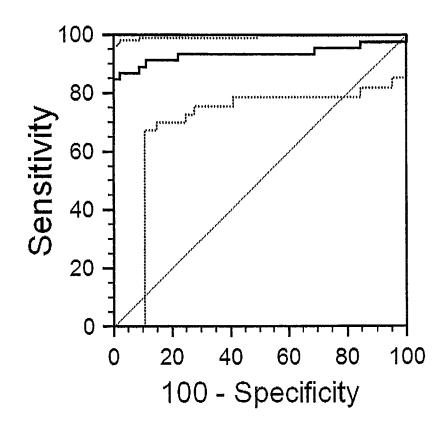


FIG. 6



METHODS AND COMPOSITIONS FOR BIOMARKERS OF FATIGUE

STATEMENT OF PRIORITY

This application is a continuation application of, and claims priority to, U.S. application Ser. No. 13/839,332, filed Mar. 15, 2013, which is a continuation-in-part application of, and claims priority to, PCT Application No. PCT/US2012/064798, filed Nov. 13, 2012, which claims the benefit, under 35 U.S.C. §119(e), of U.S. Provisional Application Ser. No. 61/559,632, filed Nov. 14, 2011, the entire contents of each of which are incorporated by reference herein.

STATEMENT REGARDING ELECTRONIC FILING OF A SEQUENCE LISTING

A Sequence Listing in ASCII text format, submitted under 37 C.F.R. §1.821, entitled 9556-4IP_ST25.txt, 187,949 bytes in size, generated on Sep. 30, 2013 and filed electronically via EFS-Web, is provided in lieu of a paper copy. This Sequence Listing is hereby incorporated by reference into the specification for its disclosures.

FIELD OF THE INVENTION

The present invention relates to biomarkers and methods of their use in identifying fatigue, disease states associated with fatigue, recovery from fatigue and/or physical performance capability in a subject.

BACKGROUND OF THE INVENTION

There is great interest in finding methods that can be used to diagnosis, evaluate and/or monitor objectively the disease 35 state referred to as chronic fatigue syndrome (CFS). As one example, an objective, saliva-based measurement tool would be useful in determining whether an individual is experiencing a level of fatigue sufficient to meet a diagnosis of chronic fatigue syndrome. Other applications include monitoring 40 changes in fatigue level, e.g., in a subject diagnosed with CFS.

The present invention provides methods and compositions for diagnosing CFS, identifying subjects having an increased risk or likelihood of having or developing CFS and/or monitoring or evaluating fatigue in a subject by detecting and/or measuring biomarkers in one or more samples from the subject.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1. For the biomarker bm_cfs_cand3, levels are greater in saliva from individuals with chronic fatigue syndrome (CFS) than in saliva from non-fatigued, control individuals (NF). The base 10 logarithm of the ion intensity, as determined by mass spectrometry, is shown as a function of patient type, i.e., CFS vs. NF. Data are shown as boxplots with the solid black line indicating the group median. The hollow box around the solid black line indicates the bounds of the data from the first to the third quartile. The whiskers indicate a distance 1.5× greater than the interquartile range from the nearest edge of the box. A non-parametric test suggested the two samples were unlikely to arise from a common distribution (Wilcoxon rank sum test, p<0.05).

FIG. 2. For the biomarker bm_cfs_cand4, levels are greater 65 in saliva from individuals with chronic fatigue syndrome (CFS) than in saliva from non-fatigued, control individuals

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(NF). The base 10 logarithm of the ion intensity, as determined by mass spectrometry, is shown as a function of patient type, i.e., CFS vs. NF. Data are shown as boxplots with the solid black line indicating the group median. The hollow box around the solid black line indicates the bounds of the data from the first to the third quartile. The whiskers indicate a distance 1.5× greater than the interquartile range from the nearest edge of the box. A non-parametric test suggested the two samples were unlikely to arise from a common distribution (Wilcoxon rank sum test, p<0.05).

FIG. 3. For the biomarker sp_6, levels are greater in saliva from individuals with chronic fatigue syndrome (CFS) than in saliva from non-fatigued, control individuals (NF). The base 10 logarithm of the ion intensity, as determined by mass spectrometry, is shown as a function of patient type, i.e., CFS vs. NF. Data are shown as boxplots with the solid black line indicating the group median. The hollow box around the solid black line indicates the bounds of the data from the first to the third quartile. The whiskers indicate a distance 1.5× greater than the interquartile range from the nearest edge of the box. A non-parametric test suggested the two samples were unlikely to arise from a common distribution (Wilcoxon rank sum test, p<0.05).

FIG. **4**. A flow chart regarding the inclusion/exclusion of patients, showing procedure for obtaining 46 and 45 CFS and control saliva samples, respectively, from 21,165 subjects.

FIG. 5. CFS biomarker levels in CFS and normal subjects. Y-axis shows thousands of intensity units corresponding to concentration of CFS biomarker peptide in saliva normalized to total protein. Each circle represents a single sample.

FIG. **6**. ROC curve for CFS salivary biomarker. Heavy solid line indicates sensitivity as a function of specificity (100-sensitivity). Dotted lines indicate 95% confidence interval. Light diagonal line indicates relationship between sensitivity and specificity if both CFS and control populations are the same. P-value associated with a comparison between the observed ROC AUC and ROC AUC=0.5 (no discrimination between diseased and normal) is <0.0001.

SUMMARY OF THE INVENTION

In some embodiments, the present invention provides a method of guiding a human subject's sleep schedule, comprising: a) measuring the concentration of a peptide selected from the group consisting of: 1) a peptide comprising the amino acid sequence PPGKPQGPPPQGGNQPQGPPPP-PGKPQ (SEQ ID NO:1), 2) a peptide comprising the amino acid sequence GNPQGPSPQGGNKPQGPPPPPGKPQ (SEQ ID NO:2), 3) a peptide comprising the amino acid 50 sequence SPPGKPQGPPQQEGNKPQGPPPPGKPQ (SEQ ID NO:3), and 4) any combination thereof, in a saliva sample taken from the subject; b) calculating the ratio of the concentration of the peptide(s) measured in (a) to the total amount of protein in the sample, according to the equation: ([PPGK-PQGPPPQGGNQPQGPPPPPGKPQ]+[GNPQGPSPQG-GNKPQGPPPPPGKPQ]+[SPPGKPQGPPQQEGNKPQG-PPPPGKPQ])/total protein (µg); c) having the subject initiate or resume a sleep schedule; d) measuring the concentration of a peptide selected from the group consisting of: 1) a peptide comprising the amino acid sequence PPGKPQGPPPQGGN-QPQGPPPPPGKPQ (SEQ ID NO:1), 2) a peptide comprising the amino acid sequence GNPQGPSPQGGNKPQGPP-PPPGKPQ (SEQ ID NO:2), 3) a peptide comprising the amino acid sequence SPPGKPQGPPQQEGNKPQGPPP-PGKPQ (SEQ ID NO:3), and 4) any combination thereof, in a saliva sample taken from the subject at one or more time points after (c), wherein the peptides of (d) are the same as the

peptides of (a); e) calculating the ratio of the concentration of the peptide(s) measured in (d) to the total amount of protein in the sample, according to the equation: ([PPGKPQGPPPQG-GNQPQGPPPPGKPQ]+[GNPQGPSPQGGNKPQGPPP-PPGKPQ]+[SPPGKPQGPPQQEGNKPQGPPPPGKPQ])/ total protein (µg); and f) guiding the subject's sleep schedule by modifying the duration of subsequent sleep periods using the subject's ratio(s) as calculated in (e), such that an increase in the ratio relative to the previous ratio leads to a subsequent increase in the duration of the subject's sleep period, and a 10 decrease in the ratio or a constant ratio relative to the previous ratio leads to no change in the duration of the subject's sleep period or a subsequent decrease in the duration of the subject's sleep period.

In further embodiments, the present invention provides a 15 method of guiding a human subject's use of a sleep enhancing material and/or sleep enhancing activity, comprising: a) measuring the concentration of a peptide selected from the group consisting of: 1) a peptide comprising the amino acid sequence PPGKPOGPPPOGGNOPOGPPPPGKPO (SEO 20 ID NO:1), 2) a peptide comprising the amino acid sequence GNPQGPSPQGGNKPQGPPPPPGKPQ (SEQ ID NO:2), 3) a peptide comprising the amino acid sequence SPPGKPQG-PPQQEGNKPQGPPPPGKPQ (SEQ ID NO:3), and 4) any combination thereof, in a saliva sample taken from the sub- 25 ject; b) calculating the ratio of the concentration of the peptide(s) measured in (a) to the total amount of protein in the sample, according to the equation: ([PPGKPQGPPPQGGN-OPOGPPPPPGKPO]+[GNPOGPSPOGGNKPOGPPPP-PGKPQ]+[SPPGKPQGPPQQEGNKPQGPPPPGKPQ])/to- 30 tal protein (µg); c) exposing the subject to the sleep enhancing material and/or sleep enhancing activity; d) measuring the concentration of a peptide selected from the group consisting of: 1) a peptide comprising the amino acid sequence PPGK-PQGPPPQGGNQPQGPPPPGKPQ (SEQ ID NO:1), 2) a 35 a peptide comprising the amino acid sequence SPPGKPQGcomprising the amino acid sequence GNPQGPSPQGGNKPQGPPPPPGKPQ (SEQ ID NO:2), 3) a peptide comprising the amino acid sequence SPPGKPQG-PPQQEGNKPQGPPPPGKPQ (SEQ ID NO:3), and 4) any combination thereof, in a saliva sample taken from the subject 40 at a time point after step (c), wherein the peptides of (d) are the same as the peptides of (a); e) calculating the ratio of the concentration of the peptide(s) measured in (d) to the total amount of protein in the sample, according to the equation: ([PPGKPQGPPPQGGNQPQGPPPPPGKPQ]+[GN-POGPSPQGGNKPQGPPPPPGKPQ]+[SPPGKPQGP-PQQEGNKPQGPPPPGKPQ])/total protein (μg); and f) guiding the subject's use of the sleep enhancing material and/or sleep enhancing activity using the subject's ratio(s) as calculated in (e), such that an increase in the ratio relative to 50 the previous ratio leads to a subsequent increase in the subject's use of the sleep enhancing material and/or sleep enhancing activity, and a decrease in the ratio or a constant ratio relative to the previous ratio leads to no change or a subsequent decrease in the subject's use of the sleep enhanc- 55

The present invention additionally provides a method of guiding a human subject's treatment of a sleep disorder, comprising: a) measuring the concentration of a peptide selected from the group consisting of: 1) a peptide comprising the 60 amino acid sequence PPGKPQGPPPQGGNQPQGPPPP-PGKPQ (SEQ ID NO:1), 2) a peptide comprising the amino sequence GNPQGPSPQGGNKPQGPPPPPGKPQ (SEQ ID NO:2), 3) a peptide comprising the amino acid sequence SPPGKPQGPPQQEGNKPQGPPPPGKPQ (SEQ 65 ID NO:3), and 4) any combination thereof, in a saliva sample taken from the subject; b) calculating the ratio of the concen-

ing material and/or sleep enhancing activity.

tration of the peptide(s) measured in (a) to the total amount of protein in the sample, according to the equation: ([PPGK-PQGPPPQGGNQPQGPPPPPGKPQ]+[GNPQGPSPQG-GNKPQGPPPPPGKPQ]+[SPPGKPQGPPQQEGNKPQG-PPPPGKPQ])/total protein (μg); c) treating the subject for the sleep disorder; d) measuring the concentration of a peptide selected from the group consisting of: 1) a peptide comprising the amino acid sequence PPGKPQGPPPQGGNQPQGPPP-PPGKPQ (SEQ ID NO:1), 2) a peptide comprising the amino sequence GNPQGPSPQGGNKPQGPPPPPGKPQ (SEQ ID NO:2), 3) a peptide comprising the amino acid sequence SPPGKPQGPPQQEGNKPQGPPPPGKPQ (SEQ ID NO:3), and 4) any combination thereof, in a saliva sample taken from the subject at a time point after step (c), wherein the peptides of (d) are the same as the peptides of (a); e) calculating the ratio of the concentration of the peptide(s) measured in (d) to the total amount of protein in the sample, according to the equation: ([PPGKPQGPPPQGGNQPQGP-PPPPGKPQ]+[GNPQGPSPQGGNKPQGPPPPPGKPQ]+ [SPPGKPOGPPOOEGNKPOGPPPPGKPO])/total protein (μg); and f) guiding the subject's treatment of the sleep disorder using the subject's ratio(s) as calculated in (e), such that an increase in the ratio relative to the previous ratio leads to a subsequent enhancement of the treatment for the sleep disorder, and a decrease in the ratio or a constant ratio relative to the previous ratio leads to no change or a subsequent reduction of the treatment for the sleep disorder.

Further provided herein is a method of identifying a substance and/or activity that enhances sleep, comprising: a) measuring the concentration of a peptide selected from the group consisting of: 1) a peptide comprising the amino acid sequence PPGKPQGPPPQGGNQPQGPPPPPGKPQ (SEQ ID NO:1), 2) a peptide comprising the amino acid sequence GNPQGPSPQGGNKPQGPPPPPGKPQ (SEQ ID NO:2), 3) PPQQEGNKPQGPPPPGKPQ (SEQ ID NO:3), and 4) any combination thereof, in a saliva sample taken from the subject; b) calculating the ratio of the concentration of the peptide(s) measured in (a) to the total amount of protein in the sample, according to the equation: ([PPGKPQGPPPQGGN-QPQGPPPPGKPQ]+[GNPQGPSPQGGNKPQGPPPP-PGKPQ]+[SPPGKPQGPPQQEGNKPQGPPPPGKPQ])/total protein (µg); c) exposing the subject to the test substance and/or test activity; d) measuring the concentration of a pep-45 tide selected from the group consisting of: 1) a peptide comprising the amino acid sequence PPGKPQGPPPQGGN-OPOGPPPPPGKPQ (SEQ ID NO:1), 2) a peptide comprising the amino acid sequence GNPQGPSPQGGNK-PQGPPPPGKPQ (SEQ ID NO:2), 3) a peptide comprising the amino acid sequence SPPGKPQGPPQQEGNKPQGPP-PPGKPQ (SEQ ID NO:3), and 4) any combination thereof, in a saliva sample taken from the subject at a time point after step (c), wherein the peptides of (d) are the same as the peptides of (a); e) calculating the ratio of the concentration of the peptide(s) measured in (d) to the total amount of protein in the sample, according to the equation: ([PPGKPQGPPPQGGN-QPQGPPPPPGKPQ]+[GNPQGPSPQGGNKPQGPPPP-PGKPQ]+[SPPGKPQGPPQQEGNKPQGPPPPGKPQ])/total protein (µg); and f) determining if the test substance and/or test activity enhances sleep using the subject's ratio(s) as calculated in (e), such that a decrease in the ratio relative to the previous ratio identifies the test substance and/or test activity as a substance and/or an activity that enhances sleep

In addition, the present invention provides a method of identifying a substance and/or activity that treats a sleep disorder, comprising: a) measuring the concentration of a peptide selected from the group consisting of: 1) a peptide

comprising the amino acid sequence PPGKPQGPPPQGGN-QPQGPPPPPGKPQ (SEQ ID NO:1), 2) a peptide comprising the amino acid sequence GNPQGPSPQGGNKPQGPP-PPPGKPQ (SEQ ID NO:2), 3) a peptide comprising the amino acid sequence SPPGKPQGPPQQEGNKPQGPPP- 5 PGKPQ (SEQ ID NO:3), and 4) any combination thereof, in a saliva sample taken from the subject; b) calculating the ratio of the concentration of the peptide(s) measured in (a) to the total amount of protein in the sample, according to the equa-([PPGKPOGPPPOGGNOPOGPPPPPGKPO]+[GN- 10 POGPSPQGGNKPQGPPPPPGKPQ]+[SPPGKPQGP-PQQEGNKPQGPPPPGKPQ])/total protein exposing the subject to the test substance and/or test activity; d) measuring the concentration of a peptide selected from the group consisting of: 1) a peptide comprising the amino acid sequence PPGKPQGPPPQGGNQPQGPPPPPGKPQ (SEQ ID NO:1), 2) a peptide comprising the amino acid sequence GNPQGPSPQGGNKPQGPPPPPGKPQ (SEQ ID NO:2), 3) a peptide comprising the amino acid sequence SPPGKPQG-PPQQEGNKPQGPPPPGKPQ (SEQ ID NO:3), and 4) any 20 combination thereof, in a saliva sample taken from the subject at a time point after step (c), wherein the peptides of (d) are the same as the peptides of (a); e) calculating the ratio of the concentration of the peptide(s) measured in (d) to the total amount of protein in the sample, according to the equation: 25 comprising: a) measuring the concentration of a peptide ([PPGKPQGPPPQGGNQPQGPPPPPGKPQ]+[GN-PQGPSPQGGNKPQGPPPPPGKPQ]+[SPPGKPQGP-PQQEGNKPQGPPPPGKPQ])/total protein (µg); and f) determining if the test substance and/or test activity treats the sleep disorder using the subject's ratio(s) as calculated in (e), 30 such that a decrease in the ratio relative to the previous ratio identifies the test substance and/or test activity as a substance

and/or an activity that treats the sleep disorder. A method is also provided herein of identifying a human subject that is sleep deprived, comprising: a) measuring the 35 concentration of a peptide selected from the group consisting of: 1) a peptide comprising the amino acid sequence PPGK-PQGPPPQGGNQPQGPPPPPGKPQ (SEQ ID NO:1), 2) a comprising the amino acid GNPQGPSPQGGNKPQGPPPPPGKPQ (SEQ ID NO:2), 3) 40 a peptide comprising the amino acid sequence SPPGKPQG-PPQQEGNKPQGPPPPGKPQ (SEQ ID NO:3), and 4) any combination thereof, in a saliva sample taken from each subject in a population of subjects that are not sleep deprived; b) calculating the ratio of the concentration of the peptide(s) 45 measured in (a) to the total amount of protein in each sample of (a), according to the equation: (IPPGKPOGPPPOGGN-QPQGPPPPPGKPQ]+[GNPQGPSPQGGNKPQGPPPP-PGKPQ]+[SPPGKPQGPPQQEGNKPQGPPPPGKPQ])/total protein (µg) to determine a ratio for each of the study 50 subjects in the population of (a); c) establishing a threshold ratio for the population of subjects of (a); d) measuring the concentration of a peptide selected from the group consisting of: 1) a peptide comprising the amino acid sequence PPGK-PQGPPPQGGNQPQGPPPPPGKPQ (SEQ ID NO:1), 2) a 55 nentide comprising the amino acid sequence GNPQGPSPQGGNKPQGPPPPPGKPQ (SEQ ID NO:2), 3) a peptide comprising the amino acid sequence SPPGKPQG-PPQQEGNKPQGPPPPGKPQ (SEQ ID NO:3), and 4) any combination thereof, in a saliva sample taken from a test 60 subject; e) calculating the ratio of the concentration of the peptide(s) measured in (d) to the total amount of protein in the sample, according to the equation: ([PPGKPQGPPPQGGN-QPQGPPPPGKPQ]+[GNPQGPSPQGGNKPQGPPPP-PGKPQ]+[SPPGKPQGPPQQEGNKPQGPPPPGKPQ])/to- 65 tal protein (µg) to determine a ratio for the test subject; and f) comparing the ratio of the test subject with the threshold ratio

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of (c), whereby a ratio of the test subject that is greater than the threshold ratio of (c) identifies the test subject as being sleep deprived.

Also provided herein is a method of identifying a human subject that is sleep deprived, comprising: a) measuring the concentration of a peptide selected from the group consisting of: 1) a peptide comprising the amino acid sequence PPGK-PQGPPPQGGNQPQGPPPPPGKPQ (SEQ ID NO:1), 2) a peptide comprising the amino acid sequence GNPQGPSPQGGNKPQGPPPPPGKPQ (SEQ ID NO:2), 3) a peptide comprising the amino acid sequence SPPGKPQG-PPQQEGNKPQGPPPPGKPQ (SEQ ID NO:3), and 4) any combination thereof, in a saliva sample taken from the subject; b) calculating the ratio of the concentration of the peptide(s) measured in (a) to the total amount of protein in the sample, according to the equation: ([PPGKPQGPPPQGGN-QPQGPPPPPGKPQ]+[GNPQGPSPQGGNKPQGPPPP-PGKPQ]+[SPPGKPQGPPQQEGNKPQGPPPPGKPQ])/total protein (µg) to determine a ratio for the subject; and c) comparing the ratio of the subject with a threshold ratio, whereby a ratio of the subject that is greater than the threshold ratio identifies the subject as sleep deprived.

In additional embodiments, the present invention provides a method of guiding a human subject's treatment of fatigue, selected from the group consisting of: 1) a peptide comprising the amino acid sequence PPGKPQGPPPQGGNQPQGPPP-PPGKPQ (SEQ ID NO:1), 2) a peptide comprising the amino acid sequence GNPOGPSPOGGNKPOGPPPPPGKPO (SEQ ID NO:2), 3) a peptide comprising the amino acid sequence SPPGKPQGPPQQEGNKPQGPPPPGKPQ (SEQ ID NO:3), and 4) any combination thereof, in a saliva sample taken from the subject; b) calculating the ratio of the concentration of the peptide(s) measured in (a) to the total amount of protein in the sample, according to the equation: ([PPGK-PQGPPPQGGNQPQGPPPPPGKPQ]+[GNPQGPSPQG-GNKPQGPPPPPGKPQ]+[SPPGKPQGPPQQEGNKPQG-PPPPGKPQ])/total protein (µg); c) treating the subject for fatigue; d) measuring the concentration of a peptide selected from the group consisting of: 1) a peptide comprising the amino acid sequence PPGKPQGPPPQGGNQPQGPPPP-PGKPQ (SEQ ID NO:1), 2) a peptide comprising the amino sequence GNPQGPSPQGGNKPQGPPPPPGKPQ (SEQ ID NO:2), 3) a peptide comprising the amino acid sequence SPPGKPQGPPQQEGNKPQGPPPPGKPQ (SEQ ID NO:3), and 4) any combination thereof, in a saliva sample taken from the subject at a time point after step (c), wherein the peptides of (d) are the same as the peptides of (a); e) calculating the ratio of the concentration of the peptide(s) measured in (d) to the total amount of protein in the sample, according to the equation: ([PPGKPQGPPPQGGNQPQGP-PPPPGKPO]+[GNPQGPSPQGGNKPQGPPPPPGKPQ]+ [SPPGKPQGPPQQEGNKPQGPPPPGKPQ])/total protein (μg); and f) guiding the subject's treatment of fatigue using the subject's ratio(s) as calculated in (e), such that a an increase in the ratio relative to the previous ratio leads to a subsequent enhancement of the treatment for fatigue, and a decrease in the ratio or a constant ratio relative to the previous ratio leads to no change or a subsequent reduction of the treatment for fatigue.

Further provided herein is a method of identifying a substance and/or activity that reduces fatigue, comprising: a) measuring the concentration of a peptide selected from the group consisting of: 1) a peptide comprising the amino acid sequence PPGKPQGPPPQGGNQPQGPPPPPGKPQ (SEQ ID NO:1), 2) a peptide comprising the amino acid sequence GNPQGPSPQGGNKPQGPPPPPGKPQ (SEQ ID NO:2), 3)

a peptide comprising the amino acid sequence SPPGKPQG-PPQQEGNKPQGPPPPGKPQ (SEQ ID NO:3), and 4) any combination thereof, in a saliva sample taken from the subject; b) calculating the ratio of the concentration of the peptide(s) measured in (a) to the total amount of protein in the 5 sample, according to the equation: ([PPGKPOGPPPOGGN-QPQGPPPPPGKPQ]+[GNPQGPSPQGGNKPQGPPPP-PGKPQ]+[SPPGKPQGPPQQEGNKPQGPPPPGKPQ])/total protein (µg); c) exposing the subject to the test substance and/or test activity; d) measuring the concentration of a pep- 10 tide selected from the group consisting of: 1) a peptide comprising the amino acid sequence PPGKPQGPPPQGGN-QPQGPPPPPGKPQ (SEQ ID NO:1), 2) a peptide comprising the amino acid sequence GNPQGPSPQGGNK-PQGPPPPGKPQ (SEQ ID NO:2), 3) a peptide comprising 15 the amino acid sequence SPPGKPQGPPQQEGNKPQGPP-PPGKPQ (SEQ ID NO:3), and 4) any combination thereof, in a saliva sample taken from the subject at a time point after step (c), wherein the peptides of (d) are the same as the peptides of (a); e) calculating the ratio of the concentration of the 20 peptide(s) measured in (d) to the total amount of protein in the sample, according to the equation: ([PPGKPQGPPPQGGN-QPQGPPPPPGKPQ]+[GNPQGPSPQGGNKPQGPPPP-PGKPQ]+[SPPGKPQGPPQQEGNKPQGPPPPGKPQ])/total protein (ug); and f) determining if the test substance and/or 25 test activity reduces fatigue using the subject's ratio(s) as calculated in (e), such that a decrease in the ratio relative to the previous ratio identifies the test substance and/or test activity as a substance and/or an activity that reduces fatigue.

Additionally provided herein is a method of guiding a 30 human subject's treatment of a chronic fatigue syndrome (CFS), comprising: a) measuring the concentration of a peptide selected from the group consisting of: 1) a peptide comprising the amino acid sequence PPGKPQGPPPQGGN-QPQGPPPPPGKPQ (SEQ ID NO:1), 2) a peptide 35 comprising the amino acid sequence GNPQGPSPQGGNK-PQGPPPPGKPQ (SEQ ID NO:2), 3) a peptide comprising the amino acid sequence SPPGKPQGPPQQEGNKPQGPP-PPGKPQ (SEQ ID NO:3), and 4) any combination thereof, in a saliva sample taken from the subject; b) calculating the ratio 40 of the concentration of the peptide(s) measured in (a) to the total amount of protein in the sample, according to the equa-([PPGKPQGPPPQGGNQPQGPPPPPGKPQ]+[GN-PQGPSPQGGNKPQGPPPPPGKPQ]+[SPPGKPQGP-PQQEGNKPQGPPPPGKPQ])/total protein (μg); c) treating 45 the subject for CFS; d) measuring the concentration of a peptide selected from the group consisting of: 1) a peptide comprising the amino acid sequence PPGKPQGPPPQGGN-QPQGPPPPPGKPQ (SEQ ID NO:1), 2) a peptide comprising the amino acid sequence GNPQGPSPQGGNKPQGPP- 50 PPPGKPQ (SEQ ID NO:2), 3) a peptide comprising the amino acid sequence SPPGKPQGPPQQEGNKPQGPPP-PGKPQ (SEQ ID NO:3), and 4) any combination thereof, in a saliva sample taken from the subject at a time point after step (c), wherein the peptides of (d) are the same as the peptides of 55 (a); e) calculating the ratio of the concentration of the peptide(s) measured in (d) to the total amount of protein in the sample, according to the equation: ([PPGKPQGPPPQGGN-QPQGPPPPPGKPQ]+[GNPQGPSPQGGNKPQGPPPP-PGKPQ]+[SPPGKPQGPPQQEGNKPQGPPPPGKPQ])/to- 60 tal protein (µg); and f) guiding the subject's treatment of CFS using the subject's ratio(s) as calculated in (e), such that an increase in the ratio relative to the previous ratio leads to a subsequent enhancement of the treatment for CFS, and a decrease in the ratio or a constant ratio relative to the previous 65 ratio leads to no change or a subsequent reduction of the treatment for CFS.

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The present invention also provides a method of identifying a substance and/or activity that treats chronic fatigue syndrome (CFS), comprising: a) measuring the concentration of a peptide selected from the group consisting of: 1) a peptide comprising the amino acid sequence PPGKPQGPPPQGGN-QPQGPPPPPGKPQ (SEQ ID NO:1), 2) a peptide comprising the amino acid sequence GNPQGPSPQGGNKPQGPP-PPPGKPQ (SEQ ID NO:2), 3) a peptide comprising the amino acid sequence SPPGKPQGPPQQEGNKPQGPPP-PGKPQ (SEQ ID NO:3), and 4) any combination thereof, in a saliva sample taken from the subject; b) calculating the ratio of the concentration of the peptide(s) measured in (a) to the total amount of protein in the sample, according to the equa-([PPGKPQGPPPQGGNQPQGPPPPPGKPQ]+[GN-PQGPSPQGGNKPQGPPPPPGKPQ]+[SPPGKPQGP-PQQEGNKPQGPPPPGKPQ])/total protein $(\mu g);$ exposing the subject to the test substance and/or test activity; d) measuring the concentration of a peptide selected from the group consisting of: 1) a peptide comprising the amino acid sequence PPGKPOGPPPOGGNOPOGPPPPPGKPO (SEO ID NO:1), 2) a peptide comprising the amino acid sequence GNPQGPSPQGGNKPQGPPPPPGKPQ (SEQ ID NO:2), 3) a peptide comprising the amino acid sequence SPPGKPQG-PPQQEGNKPQGPPPPGKPQ (SEQ ID NO:3), and 4) any combination thereof, in a saliva sample taken from the subject at a time point after step (c), wherein the peptides of (d) are the same as the peptides of (a); e) calculating the ratio of the

([PPGKPQGPPPQGGNQPQGPPPPPGKPQ]+[GN-PQGPSPQGGNKPQGPPPPPGKPQ]+[SPPGKPQGP-PQQEGNKPQGPPPPGKPQ])/total protein (μg); and f) determining if the test substance and/or test activity treats CFS using the subject's ratio(s) as calculated in (e), such that a decrease in the ratio relative to the previous ratio identifies the test substance and/or test activity as a substance and/or an activity that treats CFS.

concentration of the peptide(s) measured in (d) to the total

amount of protein in the sample, according to the equation:

Additionally provided herein is a method of identifying a human subject having an increased likelihood of having or developing chronic fatigue syndrome (CFS), comprising: a) measuring the concentration of a peptide selected from the group consisting of: 1) a peptide comprising the amino acid sequence PPGKPQGPPPQGGNQPQGPPPPPGKPQ (SEQ ID NO:1), 2) a peptide comprising the amino acid sequence GNPQGPSPQGGNKPQGPPPPPGKPQ (SEQ ID NO:2), 3) a peptide comprising the amino acid sequence SPPGKPQG-PPOOEGNKPOGPPPPGKPO (SEO ID NO:3), and 4) any combination thereof, in a saliva sample taken from each subject in a population of subjects that do not have a diagnosis of CFS or symptoms of CFS; b) calculating the ratio of the concentration of the peptide(s) measured in (a) to the total amount of protein in each sample of (a), according to the ([PPGKPQGPPPQGGNQPQGPPPPPGKPQ]+ [GNPQGPSPQGGNKPQGPPPPPGKPQ]+[SPPGKPQGP-POQEGNKPQGPPPPGKPQ])/total protein (µg) to determine a ratio for each of the study subjects in the population of (a); c) establishing a threshold ratio for the population of subjects of (a); d) measuring the concentration of a peptide selected from the group consisting of: 1) a peptide comprising the amino acid sequence PPGKPQGPPPQGGNQPQGPPP-PPGKPQ (SEQ ID NO:1), 2) a peptide comprising the amino acid sequence GNPQGPSPQGGNKPQGPPPPPGKPQ (SEQ ID NO:2), 3) a peptide comprising the amino acid sequence SPPGKPQGPPQQEGNKPQGPPPPGKPQ (SEQ ID NO:3), and 4) any combination thereof, in a saliva sample taken from a test subject; e) calculating the ratio of the concentration of the peptide(s) measured in (d) to the total

amount of protein in the sample, according to the equation: ([PPGKPQGPPPQGGNQPQGPPPPPGKPQ]+[GN-PQGPSPQGGNKPQGPPPPPGKPQ]+[SPPGKPQGP-PQQEGNKPQGPPPPGKPQ])/total protein (µg) to determine a ratio for the test subject; and f) comparing the ratio of 5 the test subject with the threshold ratio of (c), whereby a ratio of the test subject that is greater than the threshold ratio of (c) identifies the subject as having an increased likelihood of having or developing CFS.

In further aspects of this invention, a method is provided of 10 identifying a human subject having an increased likelihood of having or developing chronic fatigue syndrome (CFS), comprising: a) measuring the concentration of a peptide selected from the group consisting of: 1) a peptide comprising the amino acid sequence PPGKPQGPPPQGGNQPQGPPPP- 15 PGKPQ (SEQ ID NO:1), 2) a peptide comprising the amino sequence GNPQGPSPQGGNKPQGPPPPPGKPQ (SEQ ID NO:2), 3) a peptide comprising the amino acid sequence SPPGKPQGPPQQEGNKPQGPPPPGKPQ (SEQ ID NO:3), and 4) any combination thereof, in a saliva sample 20 taken from the subject; b) calculating the ratio of the concentration of the peptide(s) measured in (a) to the total amount of protein in the sample, according to the equation: ([PPGK-PQGPPPQGGNQPQGPPPPPGKPQ]+[GNPQGPSPQG-GNKPQGPPPPPGKPQ]+[SPPGKPQGPPQQEGNKPQG-25 PPPPGKPQ])/total protein (µg) to determine a ratio for the subject; and c) comparing the ratio of the subject with a threshold ratio, whereby a ratio of the subject that is greater than the threshold ratio identifies the subject as having an increased likelihood of having or developing CFS.

Additionally provided herein is a method of guiding a human subject's work load, comprising: a) measuring the concentration of a peptide selected from the group consisting of: 1) a peptide comprising the amino acid sequence PPGK-PQGPPPQGGNQPQGPPPPPGKPQ (SEQ ID NO:1), 2) a 35 comprising the amino acid sequence GNPQGPSPQGGNKPQGPPPPPGKPQ (SEQ ID NO:2), 3) a peptide comprising the amino acid sequence SPPGKPQG-PPQQEGNKPQGPPPPGKPQ (SEQ ID NO:3), and 4) any combination thereof, in a saliva sample taken from the sub- 40 ject; b) calculating the ratio of the concentration of the peptide(s) measured in (a) to the total amount of protein in the sample, according to the equation: ([PPGKPQGPPPQGGN-QPQGPPPPPGKPQ]+[GNPQGPSPQGGNKPQGPPPP-PGKPQ]+[SPPGKPQGPPQQEGNKPQGPPPPGKPQ])/to- 45 tal protein (µg); c) having the subject initiate or resume a work load; d) measuring the concentration of a peptide selected from the group consisting of: 1) a peptide comprising the amino acid sequence PPGKPQGPPPQGGNQPQGPPPP-PGKPQ (SEQ ID NO:1), 2) a peptide comprising the amino 50 sequence GNPQGPSPQGGNKPQGPPPPPGKPQ (SEQ ID NO:2), 3) a peptide comprising the amino acid sequence SPPGKPQGPPQQEGNKPQGPPPPGKPQ (SEQ ID NO:3), and 4) any combination thereof, in a saliva sample taken from the subject at one or more time points after (c), 55 wherein the peptides of (d) are the same as the peptides of (a); e) calculating the ratio of the concentration of the peptide(s) measured in (d) to the total amount of protein in the sample, according to the equation: ([PPGKPQGPPPQGGNQPQGP-PPPPGKPQ]+[GNPQGPSPQGGNKPQGPPPPPGKPQ]+ [SPPGKPQGPPQQEGNKPQGPPPPGKPQ])/total protein (μg); and f) guiding the subject's work load by modifying the duration of the subject's work period and/or amount of work the subject does using the subject's ratio(s) as calculated in (e), such that an increase in the ratio relative to the previous 65 ratio leads to a subsequent decrease in the duration of the subject's work period and/or a decrease in the amount of work

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the subject does, and a decrease in the ratio or a constant ratio relative to the previous ratio leads to no change in the duration of the subject's work period and/or amount of work the subject does or a subsequent increase in the duration of the subject's work period and/or amount of work the subject does.

In further aspects, the present invention provides a method of identifying a human subject sufficiently rested to carry out a work load, comprising: a) measuring the concentration of a peptide selected from the group consisting of: 1) a peptide comprising the amino acid sequence PPGKPQGPPPQGGN-QPQGPPPPPGKPQ (SEQ ID NO:1), 2) a peptide comprising the amino acid sequence GNPQGPSPQGGNKPQGPP-PPPGKPQ (SEQ ID NO:2), 3) a peptide comprising the amino acid sequence SPPGKPQGPPQQEGNKPQGPPP-PGKPQ (SEQ ID NO:3), and 4) any combination thereof, in a saliva sample taken from each subject in a population of subjects that have carried out the work load sufficiently, wherein the sample is taken from each subject at about the time the subject starts the work load; b) calculating the ratio of the concentration of the peptide(s) measured in (a) to the total amount of protein in the sample, according to the equation: ([PPGKPQGPPPQGGNQPQGPPPPPGKPQ]+[GN-

PQGPSPQGGNKPQGPPPPPGKPQ]+[SPPGKPQGP-POQEGNKPQGPPPPGKPQ])/total protein (µg) to determine a ratio for each of subject in the population of subjects of (a); c) establishing a threshold ratio for the population of subjects of (a); d) measuring the concentration of a peptide selected from the group consisting of: 1) a peptide comprising the amino acid sequence PPGKPQGPPPQGGNQPQGPPP-PPGKPQ (SEQ ID NO:1), 2) a peptide comprising the amino acid sequence GNPQGPSPQGGNKPQGPPPPPGKPQ (SEQ ID NO:2), 3) a peptide comprising the amino acid sequence SPPGKPQGPPQQEGNKPQGPPPPGKPQ (SEQ ID NO:3), and 4) any combination thereof, in a saliva sample taken from a test subject; e) calculating the ratio of the concentration of the peptide(s) measured in (a) to the total amount of protein in the sample, according to the equation: ([PPGKPQGPPPQGGNQPQGPPPPPGKPQ]+[GN-

PQGPSPQGGNKPQGPPPPPGKPQ]+[SPPGKPQGP-PQQEGNKPQGPPPPGKPQ])/total protein (μg) to determine a ratio for the test subject; and f) comparing the ratio of the test subject with the threshold ratio of (c), whereby a ratio of the test subject that is less than or equal to the threshold ratio of (c) identifies the test subject as being sufficiently rested to carry out the work load and a ratio of the test subject that is greater than the threshold ratio of (c) identifies the test subject as not being sufficiently rested to carry out the workload.

Additionally provided herein is a method of identifying a human subject that is sufficiently rested to carry out a work load, comprising: a) measuring the concentration of a peptide selected from the group consisting of: 1) a peptide comprising the amino acid sequence PPGKPQGPPPQGGNQPQGPPP-PPGKPQ (SEQ ID NO:1), 2) a peptide comprising the amino acid sequence GNPOGPSPOGGNKPOGPPPPPGKPO (SEQ ID NO:2), 3) a peptide comprising the amino acid sequence SPPGKPQGPPQQEGNKPQGPPPPGKPQ (SEQ ID NO:3), and 4) any combination thereof, in a saliva sample taken from the subject; b) calculating the ratio of the concentration of the peptide(s) measured in (a) to the total amount of protein in the sample, according to the equation: ([PPGK-PQGPPPQGGNQPQGPPPPPGKPQ]+[GNPQGPSPQG-GNKPQGPPPPPGKPQ]+[SPPGKPQGPPQQEGNKPQG-PPPPGKPQ])/total protein (µg) to determine a ratio for the subject; and c) comparing the ratio of the subject with a threshold ratio, whereby a ratio of the subject that is less than

or equal to the threshold ratio identifies the subject as being sufficiently rested to carry out the work load and a ratio of the subject that is greater than the threshold ratio identifies the subject as not being sufficiently rested to carry out the work load.

Additionally, the present invention provides a method of identifying a human subject who is fit for duty, comprising: a) measuring the concentration of a peptide selected from the group consisting of: 1) a peptide comprising the amino acid sequence PPGKPQGPPPQGGNQPQGPPPPGKPQ (SEQ ID NO:1), 2) a peptide comprising the amino acid sequence GNPQGPSPQGGNKPQGPPPPPGKPQ (SEQ ID NO:2), 3) a peptide comprising the amino acid sequence SPPGKPQG-PPQQEGNKPQGPPPPGKPQ (SEQ ID NO:3), and 4) any combination thereof, in a saliva sample taken from each sub- 15 ject in a population of subjects that are fit for duty: b) calculating the ratio of the concentration of the peptide(s) measured in (a) to the total amount of protein in the sample, according to the equation: ([PPGKPQGPPPQGGNQPQGP-PPPPGKPO1+[GNPOGPSPOGGNKPOGPPPPPGKPO1+ [SPPGKPQGPPQQEGNKPQGPPPPGKPQ])/total protein (μg) to determine a ratio for each subject in the population of subjects of (a); c) establishing a threshold ratio for the population of subjects of (a); d) measuring the concentration of a peptide selected from the group consisting of: 1) a peptide 25 comprising the amino acid sequence PPGKPQGPPPQGGN-QPQGPPPPPGKPQ (SEQ ID NO:1), 2) a peptide comprising the amino acid sequence GNPQGPSPQGGNKPQGPP-PPPGKPQ (SEQ ID NO:2), 3) a peptide comprising the amino acid sequence SPPGKPQGPPQQEGNKPQGPPP- 30 PGKPQ (SEQ ID NO:3), and 4) any combination thereof, in a saliva sample taken from a test subject; e) calculating the ratio of the concentration of the peptide(s) measured in (d) to the total amount of protein in the sample, according to the equation: ([PPGKPQGPPPQGGNQPQGPPPPPGKPQ]+ 35 [GNPQGPSPQGGNKPQGPPPPPGKPQ]+[SPPGKPQGP-PQQEGNKPQGPPPPGKPQ])/total protein (µg) to determine a ratio for the test subject; and f) comparing the ratio of the test subject with the threshold ratio of (c), whereby a ratio of the test subject that is greater than the threshold ratio of (c) 40 identifies the test subject as being not fit for duty, and a ratio of the test subject that is the same as or less than the threshold ratio of (c) identifies the test subject as being fit for duty.

Furthermore, the present invention provides a method of identifying a human subject who is fit for duty, comprising: a) 45 measuring the concentration of a peptide selected from the group consisting of: 1) a peptide comprising the amino acid sequence PPGKPQGPPPQGGNQPQGPPPPPGKPQ (SEQ ID NO:1), 2) a peptide comprising the amino acid sequence GNPQGPSPQGGNKPQGPPPPPGKPQ (SEQ ID NO:2), 3) 50 a peptide comprising the amino acid sequence SPPGKPQG-PPQQEGNKPQGPPPPGKPQ (SEQ ID NO:3), and 4) any combination thereof, in a saliva sample taken from the subject; b) calculating the ratio of the concentration of the peptide(s) measured in (a) to the total amount of protein in the 55 sample, according to the equation: ([PPGKPOGPPPOGGN-QPQGPPPPPGKPQ]+[GNPQGPSPQGGNKPQGPPPP-PGKPQ]+[SPPGKPQGPPQQEGNKPQGPPPPGKPQ])/total protein (µg) to determine a ratio for the subject; and c) comparing the ratio of the subject with a threshold ratio, 60 whereby a ratio of the subject that is greater than the threshold ratio identifies the subject as being not fit for duty, and a ratio of the subject that is the same as or less than the threshold ratio identifies the subject as being fit for duty.

In a further aspect, the present invention provides a method 65 of identifying a subject as having chronic fatigue syndrome or having an increased likelihood of having or developing

chronic fatigue syndrome, comprising: a) measuring the amount of a peptide comprising the amino acid sequence PPGKPQGPPPQGGNQPQGPPPPPGKPQ in a biological sample from each subject in a population of subjects determined not to have chronic fatigue syndrome (non-CFS subjects); b) calculating the amount of the peptide relative to the total amount of protein in each sample of (a) to determine a biomarker index for each subject in the population; c) establishing a threshold biomarker index from the biomarker indices determined in (b); d) measuring the amount of a peptide comprising the amino acid sequence PPGKPQGPPPQGGN-QPQGPPPPPGKPQ in a biological sample from a test subject; and e) calculating the amount of the peptide relative to the total amount of protein in the sample of (d) to determine a biomarker index for the test subject, wherein a biomarker index of the test subject that is higher than the threshold biomarker index of (c) identifies the subject as having chronic fatigue syndrome or having an increased likelihood of having

or developing chronic fatigue syndrome.

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In an additional aspect, the present invention provides a method of identifying a subject as having chronic fatigue syndrome or having an increased likelihood of having or developing chronic fatigue syndrome, comprising: a) measuring the amount of a peptide comprising the amino acid sequence GNPQGPSPQGGNKPQGPPPPPGKPQ in a biological sample from each subject in a population of subjects determined not to have chronic fatigue syndrome (e.g., non-CFS subjects); b) calculating the amount of the peptide relative to the total amount of protein in each sample of (a) to determine a biomarker index for each subject in the population; c) establishing a threshold biomarker index from the biomarker indices determined in (b); d) measuring the amount of a peptide comprising the amino acid sequence GNPQGPSPQGGNKPQGPPPPPGKPQ in a biological sample from a test subject; and e) calculating the amount of the peptide relative to the total amount of protein in the sample of (d) to determine a biomarker index for the test subject, wherein a biomarker index of the test subject that is higher than the threshold biomarker index of (c) identifies the subject as having chronic fatigue syndrome or having an increased likelihood of having or developing chronic fatigue syndrome.

A further aspect of this invention is a method of identifying a subject as having chronic fatigue syndrome or having an increased likelihood of having or developing chronic fatigue syndrome, comprising: a) measuring the amount of a peptide comprising the amino acid sequence SPPGKPOGPPOOEG-NKPQGPPPPGKPQ in a biological sample from each subject in a population of subjects determined not to have chronic fatigue syndrome (e.g., non-CFS subjects); b) calculating the amount of the peptide relative to the total amount of protein in each sample of (a) to determine a biomarker index for each subject in the population; c) establishing a threshold biomarker index from the biomarker indices determined in (b); d) measuring the amount of a peptide comprising the amino acid sequence SPPGKPQGPPQQEGNKPQGPPPPGKPQ in a biological sample from a test subject; and e) calculating the amount of the peptide relative to the total amount of protein in the sample of (d) to determine a biomarker index for the test subject, wherein a biomarker index of the test subject that is higher than the threshold biomarker index of (c) identifies the subject as having chronic fatigue syndrome or having an increased likelihood of having or developing chronic fatigue syndrome.

Further provided herein is a method of identifying a subject as having chronic fatigue syndrome or having an increased likelihood of having or developing chronic fatigue syndrome,

comprising: a) measuring the amount of each of 1) a peptide comprising the amino acid sequence PPGKPQGPPPQGGN-QPQGPPPPPGKPQ, 2) a peptide comprising the amino acid sequence GNPQGPSPQGGNKPQGPPPPPGKPQ, and 3) a peptide comprising the amino acid sequence SPPGKPQGP- 5 PQQEGNKPQGPPPPGKPQ in a biological sample from each subject in a population of subjects determined not to have chronic fatigue syndrome (e.g., non-CFS subjects); b) calculating the amount of the peptides relative to the total amount of protein in each sample of (a) to determine a biom- 10 arker index for each subject of the population; c) establishing a threshold biomarker index from the biomarker indices determined in (b); d) measuring the amount of each of 1) a peptide comprising the amino acid sequence PPGKPQGPP-PQGGNQPQGPPPPPGKPQ, 2) a peptide comprising the 15 amino acid sequence GNPQGPSPQGGNKPQGPPPP-PGKPQ, and 3) a peptide comprising the amino acid sequence SPPGKPQGPPQQEGNKPQGPPPPGKPQ in a biological sample from a test subject; and e) calculating the amount of the peptides relative to the total amount of protein 20 in the sample of (d) to determine a biomarker index for the test subject, wherein a biomarker index of the test subject that is higher than the threshold biomarker index of (c) identifies the subject as having chronic fatigue syndrome or having an increased likelihood of having or developing chronic fatigue 25

Also provided herein is a method of identifying a subject as having chronic fatigue syndrome or having an increased likelihood of having or developing chronic fatigue syndrome, comprising: a) measuring the amount of two or more peptides 30 selected from the group consisting of: 1) a peptide comprising the amino acid sequence PPGKPQGPPPQGGNQPQGPPP-PPGKPQ, 2) a peptide comprising the amino acid sequence GNPQGPSPQGGNKPQGPPPPPGKPQ, and 3) a peptide comprising the amino acid sequence SPPGKPQGPPQQEG- 35 NKPQGPPPPGKPQ, in a biological sample from each subject in a population of subjects determined not to have chronic fatigue syndrome; b) calculating the amount of the peptides relative to the total amount of protein in each sample of (a) to determine a biomarker index for each subject of the popula- 40 tion; c) establishing a threshold biomarker index from the biomarker indices determined in (b); d) measuring the amount of two or more peptides selected from the group consisting of: 1) a peptide comprising the amino acid sequence PPGKPQGPPPQGGNQPQGPPPPGKPQ, 2) a 45 peptide comprising the amino acid sequence GNPOGPSPOGGNKPQGPPPPPGKPQ, and 3) a peptide comprising the amino acid sequence SPPGKPQGPPQQEG-NKPQGPPPPGKPQ in a biological sample from a test subject (e.g., wherein the peptides of (d) are the same as peptides 50 as measured in (a)); and e) calculating the amount of the peptides relative to the total amount of protein in the sample of (d) to determine a biomarker index for the test subject, wherein a biomarker index of the test subject that is higher than the threshold biomarker index of (c) identifies the subject 55 as having chronic fatigue syndrome or having an increased likelihood of having or developing chronic fatigue syndrome.

An additional aspect of this invention includes a method of identifying a subject as having chronic fatigue syndrome or having an increased likelihood of having or developing 60 chronic fatigue syndrome, comprising: a) measuring the amount of human basic proline-rich protein 1 (PRB1) in a biological sample from each subject in a population of subjects determined not to have chronic fatigue syndrome (e.g., non-CFS subjects); b) calculating the amount of PRB1 relative to the total amount of protein in each sample of (a) to determine a biomarker index for each subject in the popula-

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tion; c) establishing a threshold biomarker index from the biomarker indices determined in (b); d) measuring the amount of human basic proline-rich protein 1 (PRB1) in a biological sample from a test subject; and e) calculating the amount of PRB1 relative to the total amount of protein in the sample of (d) to determine a biomarker index for the test subject, wherein a biomarker index of the test subject that is higher than the threshold biomarker index of (c) identifies the subject as having chronic fatigue syndrome or having an increased likelihood of having or developing chronic fatigue syndrome.

Other aspects of this invention include a method of identifying a subject as having chronic fatigue syndrome or having an increased likelihood of having or developing chronic fatigue syndrome, comprising: a) measuring the amount of human basic proline-rich protein 2 (PRB2) in a biological sample from each subject in a population of subjects determined not to have chronic fatigue syndrome (e.g., non-CFS subjects); b) calculating the amount of PRB2 relative to the total amount of protein in each sample of (a) to determine a biomarker index for each subject in the population; c) establishing a threshold biomarker index from the biomarker indices determined in (b); d) measuring the amount of human basic proline-rich protein 2 (PRB2) in a biological sample from a test subject; and e) calculating the amount of PRB2 relative to the total amount of protein in the sample of (d) to determine a biomarker index for the test subject, wherein a biomarker index of the test subject that is higher than the threshold biomarker index of (c) identifies the subject as having chronic fatigue syndrome or having an increased likelihood of having or developing chronic fatigue syndrome.

The present invention also provides a method of identifying a subject as having chronic fatigue syndrome or having an increased likelihood of having or developing chronic fatigue syndrome, comprising: a) measuring the amount of human basic proline-rich protein 4 (PRB4) in a biological sample from each subject in a population of subjects determined not to have chronic fatigue syndrome (e.g., non-CFS subjects); b) calculating the amount of PRB4 relative to the total amount of protein in each sample of (a) to determine a biomarker index for each subject in the population; c) establishing a threshold biomarker index from the biomarker indices determined in (b); d) measuring the amount of human basic proline-rich protein 4 (PRB4) in a biological sample from a test subject; and e) calculating the amount of PRB4 relative to the total amount of protein in the sample of (d) to determine a biomarker index for the test subject, wherein a biomarker index of the test subject that is higher than the threshold biomarker index of (c) identifies the subject as having chronic fatigue syndrome or having an increased likelihood of having or developing chronic fatigue syndrome.

Furthermore, the present invention provides a method of identifying a subject as having chronic fatigue syndrome or having an increased likelihood of having or developing chronic fatigue syndrome, comprising: a) measuring the amount of each of 1) human basic proline-rich protein 1 (PRB1), 2) human basic proline-rich protein 2 (PRB2), and 3) human basic proline-rich protein 4 (PRB4) in a biological sample from each subject in a population of subjects determined not to have chronic fatigue syndrome (e.g., non-CFS subjects); b) calculating the amount of the proteins relative to the total amount of protein in each sample of (a) to determine a biomarker index for each subject in the population; c) establishing a threshold biomarker index from the biomarker indices determined in (b); d) measuring the amount of 1) human basic proline-rich protein 1 (PRB1), 2) human basic prolinerich protein 2 (PRB2), and 3) human basic proline-rich pro-

tein 4 (PRB4) in a biological sample from a test subject; and e) calculating the amount of the proteins relative to the total amount of protein in the sample of (d) to determine a biomarker index for the test subject, wherein a biomarker index of the test subject that is higher than the threshold biomarker index of (c) identifies the subject as having chronic fatigue syndrome or having an increased likelihood of having or developing chronic fatigue syndrome.

Additionally provided herein is a method of identifying a subject as having chronic fatigue syndrome or having an increased likelihood of having or developing chronic fatigue syndrome, comprising: a) measuring the amount of two or more proteins selected from the group consisting of 1) human basic proline-rich protein 1 (PRB1), 2) human basic prolinerich protein 2 (PRB2), and 3) human basic proline-rich pro- 15 tein 4 (PRB4), in a biological sample from each subject in a population of subjects determined not to have chronic fatigue syndrome; b) calculating the amount of the proteins relative to the total amount of protein in each sample of (a) to determine a biomarker index for each subject in the population; c) 20 establishing a threshold biomarker index from the biomarker indices determined in (b); d) measuring the amount of two or more proteins selected from the group consisting of: 1) human basic proline-rich protein 1 (PRB1), 2) human basic proline-rich protein 2 (PRB2), and 3) human basic proline- 25 rich protein 4 (PRB4), in a biological sample from a test subject, (e.g., wherein the proteins of (d) are the same proteins as measured in (a)); and e) calculating the amount of the proteins relative to the total amount of protein in the sample of (d) to determine a biomarker index for the test subject, 30 wherein a biomarker index of the test subject that is higher than the threshold biomarker index of (c) identifies the subject as having chronic fatigue syndrome or having an increased likelihood of having or developing chronic fatigue syndrome.

In further embodiments, the present invention provides a 35 method of identifying a subject as having chronic fatigue syndrome or having an increased likelihood of having or developing chronic fatigue syndrome, comprising: a) measuring the amount of a peptide comprising the amino acid sequence PPGKPQGPPPQGGNQPQGPPPPGKPQ in a 40 biological sample from a test subject; and b) calculating the amount of the peptide relative to the total amount of protein in the sample of (a) to determine a biomarker index for the test subject, wherein a biomarker index of the test subject that is higher than a threshold biomarker index identifies the subject as having chronic fatigue syndrome or having an increased likelihood of having or developing chronic fatigue syndrome.

In addition, the present invention provides a method of identifying a subject as having chronic fatigue syndrome or having an increased likelihood of having or developing 50 chronic fatigue syndrome, comprising: a) measuring the amount of a peptide comprising the amino acid sequence GNPQGPSPQGGNKPQGPPPPPGKPQ in a biological sample from a test subject; and b) calculating the amount of the peptide relative to the total amount of protein in the 55 sample of (a) to determine a biomarker index for the test subject, wherein a biomarker index of the test subject that is higher than a threshold biomarker index identifies the subject as having chronic fatigue syndrome or having an increased likelihood of having or developing chronic fatigue syndrome. 60

Also provided herein is a method of identifying a subject as having chronic fatigue syndrome or having an increased likelihood of having or developing chronic fatigue syndrome, comprising: a) measuring the amount of a peptide comprising the amino acid sequence SPPGKPQGPPQQEGNKPQGPP-65 PPGKPQ in a biological sample from a test subject; and b) calculating the amount of the peptide relative to the total

amount of protein in the sample of (a) to determine a biomarker index for the test subject, wherein a biomarker index of the test subject that is higher than a threshold biomarker index identifies the subject as having chronic fatigue syndrome or having an increased likelihood of having or developing chronic fatigue syndrome.

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Furthermore, the present invention provides a method of identifying a subject as having chronic fatigue syndrome or having an increased likelihood of having or developing chronic fatigue syndrome, comprising: a) measuring the amount of 1) a peptide comprising the amino acid sequence PPGKPQGPPPQGGNQPQGPPPPPGKPQ, 2) a peptide comprising the amino acid sequence GNPQGPSPQGGNK-PQGPPPPGKPQ, and 3) a peptide comprising the amino acid sequence SPPGKPQGPPQQEGNKPQGPPPPGKPQ in a biological sample from a test subject; and b) calculating the amount of the peptides relative to the total amount of protein in the sample of (a) to determine a biomarker index for the test subject, wherein a biomarker index of the test subject that is higher than a threshold biomarker index identifies the subject as having chronic fatigue syndrome or having an increased likelihood of having or developing chronic fatigue syndrome.

The present invention also provides a method of identifying a subject as having chronic fatigue syndrome or having an increased likelihood of having or developing chronic fatigue syndrome, comprising: a) measuring the amount of two or more peptides selected from the group consisting of: 1) a peptide comprising the amino acid sequence PPGKPQGPP-PQGGNQPQGPPPPPGKPQ, 2) a peptide comprising the amino acid sequence GNPQGPSPQGGNKPQGPPPP-PGKPQ, and 3) a peptide comprising the amino acid sequence SPPGKPQGPPQQEGNKPQGPPPPGKPQ, in a biological sample from a test subject; and b) calculating the amount of the peptides relative to the total amount of protein in the sample of (a) to determine a biomarker index for the test subject, wherein a biomarker index of the test subject that is higher than a threshold biomarker index identifies the subject as having chronic fatigue syndrome or having an increased likelihood of having or developing chronic fatigue syndrome.

Additionally, the present invention provides a method of identifying a subject as having chronic fatigue syndrome or having an increased likelihood of having or developing chronic fatigue syndrome, comprising: a) measuring the amount of human basic proline-rich protein 1 (PRB1) in a biological sample from a test subject; and b) calculating the amount of PRB1 relative to the total amount of protein in the sample of (a) to determine a biomarker index for the test subject, wherein a biomarker index of the test subject that is higher than a threshold biomarker index identifies the subject as having chronic fatigue syndrome or having an increased likelihood of having or developing chronic fatigue syndrome.

In yet further embodiments, the present invention provides a method of identifying a subject as having chronic fatigue syndrome or having an increased likelihood of having or developing chronic fatigue syndrome, comprising: a) measuring the amount of human basic proline-rich protein 2 (PRB2) in a biological sample from a test subject; and b) calculating the amount of PRB2 relative to the total amount of protein in the sample of (a) to determine a biomarker index for the test subject, wherein a biomarker index of the test subject that is higher than a threshold biomarker index identifies the subject as having chronic fatigue syndrome or having an increased likelihood of having or developing chronic fatigue syndrome.

Further provided herein is a method of identifying a subject as having chronic fatigue syndrome or having an increased likelihood of having or developing chronic fatigue syndrome,

comprising: a) measuring the amount of human basic prolinerich protein 4 (PRB4) in a biological sample from a test subject; and b) calculating the amount of PRB4 relative to the total amount of protein in the sample of (a) to determine a biomarker index for the test subject, wherein a biomarker 5 index of the test subject that is higher than a threshold biomarker index identifies the subject as having chronic fatigue syndrome or having an increased likelihood of having or developing chronic fatigue syndrome.

The present invention also provides a method of identifying a subject as having chronic fatigue syndrome or having an increased likelihood of having or developing chronic fatigue syndrome, comprising: a) measuring the amount of 1) human basic proline-rich protein 1 (PRB1), 2) human basic prolinerich protein 2 (PRB2), and 3) human basic proline-rich pro- 15 tein 4 (PRB4) in a biological sample from a test subject; and b) calculating the amount of the proteins relative to the total amount of protein in the sample of (a) to determine a biomarker index for the test subject, wherein a biomarker index of the test subject that is higher than a threshold biomarker index 20 identifies the subject as having chronic fatigue syndrome or having an increased likelihood of having or developing chronic fatigue syndrome.

Furthermore, the present invention provides a method of identifying a subject as having chronic fatigue syndrome or 25 having an increased likelihood of having or developing chronic fatigue syndrome, comprising: a) measuring the amount of two or more proteins selected from the group consisting of: 1) human basic proline-rich protein 1 (PRB1), 2) human basic proline-rich protein 2 (PRB2), and 3) human 30 basic proline-rich protein 4 (PRB4), in a biological sample from a test subject; and b) calculating the amount of the proteins relative to the total amount of protein in the sample of (a) to determine a biomarker index for the test subject, wherein a biomarker index of the test subject that is higher 35 than a threshold biomarker index identifies the subject as having chronic fatigue syndrome or having an increased likelihood of having or developing chronic fatigue syndrome.

Additional aspects of this invention include a method of identifying a decrease over time in fatigue level of a subject, 40 comprising: a) measuring, at a first time point, an amount of a peptide comprising the amino acid sequence PPGKPQGP-PPQGGNQPQGPPPPPGKPQ in a first biological sample from the subject; b) calculating the amount of the peptide of (a) relative to the total amount of protein in the first sample to 45 determine a first biomarker index for the subject at the first time point; c) measuring, at a second or subsequent time point(s), an amount of the peptide comprising an amino acid sequence PPGKPQGPPPQGGNQPQGPPPPGKPQ, in a second or subsequent biological sample(s); and d) calculating 50 the amount of the peptide of (c) relative to the total amount of the protein in the second or subsequent sample(s) to determine a second or subsequent biomarker index for the subject at the second or subsequent time point(s), wherein a decrease in the second or subsequent biomarker index relative to the 55 identifying a decrease over time in fatigue level of a subject, first biomarker index identifies a decrease over time in the fatigue level of the subject.

The present invention further provides a method of identifying a decrease over time in fatigue level of a subject, comprising: a) measuring, at a first time point, an amount of a 60 comprising the amino acid sequence GNPQGPSPQGGNKPQGPPPPPGKPQ, in a first biological sample from the subject; b) calculating the amount of the peptide of (a) relative to the total amount of protein in the first sample to determine a first biomarker index for the subject at 65 the first time point; c) measuring, at a second or subsequent time point(s), an amount of the peptide comprising an amino

acid sequence GNPQGPSPQGGNKPQGPPPPPGKPQ in a second or subsequent biological sample(s); and d) calculating the amount of the peptide of (c) relative to the total amount of the protein in the second or subsequent sample(s) to determine a second or subsequent biomarker index for the subject at the second or subsequent time point(s), wherein a decrease in the second or subsequent biomarker index relative to the first biomarker index identifies a decrease over time in the fatigue level of the subject.

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Also provided herein is a method of identifying a decrease over time in fatigue level of a subject, comprising: a) measuring, at a first time point, an amount of a peptide comprising the amino acid sequence SPPGKPQGPPQQEGNKPQGPP-PPGKPQ in a first biological sample from the subject; b) calculating the amount of the peptide of (a) relative to the total amount of protein in the first sample to determine a first biomarker index for the subject at the first time point; c) measuring, at a second or subsequent time point(s), an amount of the peptide comprising an amino acid sequence SPPGKPQGPPQQEGNKPQGPPPPGKPQ in a second or subsequent biological sample(s); and d) calculating the amount of the peptide of (c) relative to the total amount of the protein in the second or subsequent sample(s) to determine a second or subsequent biomarker index for the subject at the second or subsequent time point(s), wherein a decrease in the second or subsequent biomarker index relative to the first biomarker index identifies a decrease over time in the fatigue level of the subject.

Furthermore, the present invention provides a method of identifying a decrease over time in fatigue level of a subject, comprising: a) measuring, at a first time point, an amount of 1) a peptide comprising the amino acid sequence PPGKPQG-PPPQGGNQPQGPPPPPGKPQ, 2) a peptide comprising the amino acid sequence GNPQGPSPQGGNKPQGPPPP-PGKPQ, and 3) a peptide comprising the amino acid sequence SPPGKPQGPPQQEGNKPQGPPPPGKPQ in a first biological sample from the subject; b) calculating the amount of the peptides of (a) relative to the total amount of protein in the first sample to determine a first biomarker index for the subject at the first time point; c) measuring, at a second or subsequent time point(s), an amount of 1) a peptide comprising the amino acid sequence PPGKPQGPPPQGGN-QPQGPPPPPGKPQ, 2) a peptide comprising the amino acid sequence GNPQGPSPQGGNKPQGPPPPPGKPQ, and 3) a peptide comprising the amino acid sequence SPPGKPQGP-PQQEGNKPQGPPPPGKPQ in a second or subsequent biological sample(s); and d) calculating the amount of the peptides of (c) relative to the total amount of the protein in the respective sample(s) to determine a second or subsequent biomarker index for the subject at the second or subsequent time point(s), wherein a decrease in the second or subsequent biomarker index relative to the first biomarker index identifies a decrease over time in the fatigue level of the subject.

Furthermore, the present invention provides a method of comprising: a) measuring, at a first time point, an amount of two or more peptides selected from the group consisting of: 1) a peptide comprising the amino acid sequence PPGKPQGP-PPQGGNQPQGPPPPGKPQ, 2) a peptide comprising the amino acid sequence GNPQGPSPQGGNKPQGPPPP-PGKPQ, and 3) a peptide comprising the amino acid sequence SPPGKPQGPPQQEGNKPQGPPPPGKPQ in a first biological sample from the subject; b) calculating the amount of the peptides of (a) relative to the total amount of protein in the first sample to determine a first biomarker index for the subject at the first time point; c) measuring, at a second or subsequent time point(s), an amount of 1) a peptide com-

prising the amino acid sequence PPGKPQGPPPQGGN-QPQGPPPPPGKPQ, 2) a peptide comprising the amino acid sequence GNPQGPSPQGGNKPQGPPPPPGKPQ, and 3) a peptide comprising the amino acid sequence SPPGKPQGP-PQQEGNKPQGPPPPGKPQ in a second or subsequent bio- 5 logical sample(s) (e.g., wherein the peptides measured in (c) are the same peptides as measured in (a)); and d) calculating the amount of the peptides of (c) relative to the total amount of the protein in the respective sample(s) to determine a second or subsequent biomarker index for the subject at the second or subsequent time point(s), wherein a decrease in the second or subsequent biomarker index relative to the first biomarker index identifies a decrease over time in the fatigue level of the subject.

In an additional aspect, the present invention provides a 15 method of identifying a decrease over time in fatigue level of a subject, comprising: a) measuring, at a first time point, an amount of human basic proline-rich protein 1 (PRB1) in a first biological sample from the subject; b) calculating the amount of the protein of (a) relative to the total amount of protein in 20 the first sample to determine a first biomarker index for the subject at the first time point; c) measuring, at a second or subsequent time point(s), an amount of human basic prolinerich protein 1 (PRB1) in a second or subsequent biological sample(s); and d) calculating the amount of the protein of (c) 25 relative to the total amount of the protein in the second or subsequent sample(s) to determine a second or subsequent biomarker index for the subject at the second or subsequent time point(s), wherein a decrease in the second or subsequent biomarker index relative to the first biomarker index identifies 30 a decrease over time in the fatigue level of the subject.

In a further aspect, the present invention provides a method of identifying a decrease over time in fatigue level of a subject, comprising: a) measuring, at a first time point, an amount of human basic proline-rich protein 2 (PRB2) in a first bio- 35 logical sample from the subject; b) calculating the amount of the protein of (a) relative to the total amount of protein in the first sample to determine a first biomarker index for the subject at the first time point; c) measuring, at a second or subsequent time point(s), an amount of human basic proline-rich 40 protein 2 (PRB2) in a second or subsequent biological sample(s); and d) calculating the amount of the protein of (c) relative to the total amount of the protein in the second or subsequent sample(s) to determine a second or subsequent time point(s), wherein a decrease in the second or subsequent biomarker index relative to the first biomarker index identifies a decrease over time in the fatigue level of the subject.

Further provided herein is a method of identifying a decrease over time in fatigue level of a subject, comprising: a) 50 measuring, at a first time point, an amount of human basic proline-rich protein 4 (PRB4) in a first biological sample from the subject; b) calculating the amount of the protein of (a) relative to the total amount of protein in the first sample to determine a first biomarker index for the subject at the first 55 time point; c) measuring, at a second or subsequent time point(s), an amount of human basic proline-rich protein 4 (PRB4) in a second or subsequent biological sample(s); and d) calculating the amount of the protein of (c) relative to the total amount of the protein in the second or subsequent 60 sample(s) to determine a second or subsequent biomarker index for the subject at the second or subsequent time point(s), wherein a decrease in the second or subsequent biomarker index relative to the first biomarker index identifies a decrease over time in the fatigue level of the subject.

In an additional aspect of this invention, a method is provided, of identifying a decrease over time in fatigue level of a 20

subject, comprising: a) measuring, at a first time point, an amount of 1) human basic proline-rich protein 1 (PRB1), 2) human basic proline-rich protein 2 (PRB2), and 3) human basic proline-rich protein 4 (PRB4) in a first biological sample from the subject; b) calculating the amount of the proteins of (a) relative to the total amount of protein in the first sample to determine a first biomarker index for the subject at the first time point; c) measuring, at a second or subsequent time point(s), an amount of 1) human basic proline-rich protein 1 (PRB1), 2) human basic proline-rich protein 2 (PRB2), and 3) human basic proline-rich protein 4 (PRB4) in a second or subsequent biological sample(s); and d) calculating the amount of the proteins of (c) relative to the total amount of the protein in the second or subsequent sample(s) to determine a second or subsequent biomarker index for the subject at the second or subsequent time point(s), wherein a decrease in the second or subsequent biomarker index relative to the first biomarker index identifies a decrease over time in the fatigue level of the subject.

In a further aspect of this invention, a method is provided. of identifying a decrease over time in fatigue level of a subject, comprising: a) measuring, at a first time point, an amount of two or more proteins selected from the group consisting of: 1) human basic proline-rich protein 1 (PRB1), 2) human basic proline-rich protein 2 (PRB2), and 3) human basic prolinerich protein 4 (PRB4) in a first biological sample from the subject; b) calculating the amount of the proteins of (a) relative to the total amount of protein in the first sample to determine a first biomarker index for the subject at the first time point; c) measuring, at a second or subsequent time point(s), an amount of two or more proteins selected from the group consisting of: 1) human basic proline-rich protein 1 (PRB1), 2) human basic proline-rich protein 2 (PRB2), and 3) human basic proline-rich protein 4 (PRB4) in a second or subsequent biological sample(s) (e.g., wherein the peptides measured in (c) are the same peptides as measured in (a); and d) calculating the amount of the proteins of (c) relative to the total amount of the protein in the second or subsequent sample(s) to determine a second or subsequent biomarker index for the subject at the second or subsequent time point(s), wherein a decrease in the second or subsequent biomarker index relative to the first biomarker index identifies a decrease over time in the fatigue level of the subject.

In yet further embodiments, the present invention provides biomarker index for the subject at the second or subsequent 45 a method of guiding a human subject's treatment for chronic fatigue syndrome (CFS), comprising: a) measuring the concentration of a peptide selected from the group consisting of 1) a peptide comprising the amino acid sequence PPGKPQG-PPPQGGNQPQGPPPPPGKPQ (SEQ ID NO:1), 2) a peptide comprising the amino acid sequence GNPQGPSPQGGNK-PQGPPPPGKPQ (SEQ ID NO:2), 3) a peptide comprising the amino acid sequence SPPGKPQGPPQQEGNKPQGPP-PPGKPQ, (SEQ ID NO:3), and 4) any combination thereof, in a saliva sample taken from the subject; b) identifying the subject's level of chronic fatigue by calculating the ratio of the concentration of the peptide(s) measured in (a) to the total amount of protein in the sample, according to the equation: ([PPGKPQGPPPQGGNQPQGPPPPPGKPQ]+[GN-

PQGPSPQGGNKPQGPPPPPGKPQ]+[SPPGKPQGP-

PQQEGNKPQGPPPPGKPQ])/total protein (µg); c) having the subject initiate or resume a treatment program for chronic fatigue syndrome; d) measuring the concentration of a peptide selected from the group consisting of 1) a peptide comprising the amino acid sequence PPGKPQGPPPQGGN-QPQGPPPPPGKPQ (SEQ ID NO:1), 2) a peptide comprising the amino acid sequence GNPQGPSPQGGNK-PQGPPPPGKPQ (SEQ ID NO:2), 3) a peptide comprising

the amino acid sequence SPPGKPQGPPQQEGNKPQGPP-PPGKPQ, (SEQ ID NO:3), and 4) any combination thereof, in a saliva sample taken from the subject at one or more time points after (c), wherein the peptides of (d) are the same as the peptides of (a); e) identifying the subject's level of chronic fatigue by calculating the ratio of the concentration of the peptide(s) measured in (d) to the total amount of protein in the sample of (d) according to the equation: ([PPGKPQGPP-PQGGNQPQGPPPPGKPQ]+[GNPQGPSPQGGNKPQG-PPPPGKPQ]+[SPPGKPQGPPQQEGNKPQGPPP-10

PGKPQ])/total protein (µg); and f) guiding the subject's treatment for chronic fatigue syndrome by modifying the intensity of subsequent treatments using the latest of the subject's ratio(s) as calculated in (e), such that an increase in chronic fatigue relative to the previous measurement of 15 chronic fatigue leads to a subsequent increase in the subject's treatment intensity.

Also provided herein is a method of evaluating the effect of a treatment material and/or activity on the chronic fatigue level of a human subject (e.g., a subject diagnosed as having, 20 determined to have or suspected of having chronic fatigue syndrome (CFS), comprising: a) measuring the concentration of a peptide selected from the group consisting of 1) a peptide comprising the amino acid sequence PPGKPQGPPPQGGN-OPOGPPPPPGKPQ (SEQ ID NO:1), 2) a peptide compris- 25 ing the amino acid sequence GNPQGPSPQGGNKPQGPP-PPPGKPQ (SEQ ID NO:2), a 3) a peptide comprising the amino acid sequence SPPGKPQGPPQQEGNKPQGPPP-PGKPQ (SEQ ID NO:3), and 4) any combination thereof, in a saliva sample taken from a subject at a time point prior to 30 exposing the subject to a treatment material and/or activity; b) identifying the subject's level of chronic fatigue by calculating the ratio of the concentration of the peptide(s) measured in (a) to the total amount of protein in the sample, according to equation: ([PPGKPQGPPPQGGNQPQGPPPP- 35 PGKPQ]+[GNPQGPSPQGGNKPQGPPPPPGKPQ]+[SP-PGKPQGPPQQEGNKPQGPPPPGKPQ])/total (μg); c) exposing the subject to the treatment material and/or performance enhancing activity; d) measuring the concentration of a peptide selected from the group consisting of: 1) a 40 peptide comprising the amino acid sequence PPGKPQGPP-PQGGNQPQGPPPPPGKPQ (SEQ ID NO:1), 2) a peptide comprising the amino acid sequence GNPQGPSPQGGNK-PQGPPPPPGKPQ (SEQ ID NO:2), a 3) a peptide comprising the amino acid sequence SPPGKPQGPPQQEGNKPQGPP- 45 PPGKPQ (SEQ ID NO:3), and 4) any combination thereof, in a saliva sample taken from the subject at one or more time points after (c), wherein the peptides of (d) are the same as the peptides of (a); e) identifying the subject's level of chronic fatigue by calculating the ratio of the concentration of the 50 peptide(s) measured in (d) to the total amount of protein in the sample, according to the equation: ([PPGKPQGPPPQGGN-QPQGPPPPGKPQ]+[GNPQGPSPQGGNKPQGPPPP-PGKPQ]+[SPPGKPQGPPQQEGNKPQGPPPPGKPQ])/total protein (µg); and f) evaluating the effect of the treatment 55 material and/or activity on the chronic fatigue status of the subject by comparing the ratios of (b) and (e), wherein an increase in the ratio of (e) relative to the ratio of (b) is indicative of increased chronic fatigue of the subject, a decrease in the ratio of (e) relative to the ratio of (b) is indicative of 60 decreased chronic fatigue of the subject, and a constant ratio of (e) relative to the ratio of (b) is indicative of no change in chronic fatigue of the subject.

Additionally provided herein is a method of guiding a human subject's physical training activity, comprising: a) 65 measuring the concentration of a peptide selected from the group consisting of: 1) a peptide comprising the amino acid

ID NO:1), 2) a peptide comprising the amino acid sequence GNPQGPSPQGGNKPQGPPPPPGKPQ (SEQ ID NO:2), a 3) a peptide comprising the amino acid sequence SPPGK-PQGPPQQEGNKPQGPPPPGKPQ (SEQ ID NO:3), and 4) any combination thereof, in a saliva sample taken from the subject when the subject is in a rested state, wherein the subject is an adult athlete or an amateur athlete; b) identifying

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sequence PPGKPOGPPPOGGNOPOGPPPPPGKPO (SEO

subject is an adult athlete or an amateur athlete; b) identifying the subject's level of physical fatigue by calculating the ratio of the concentration of the peptide(s) measured in (a) to the total amount of protein in the sample, according to the equation:

([PPGKPQGPPPQGGNQPQGPPPPPGKPQ]+[GN-

PQGPSPQGGNKPQGPPPPPGKPQ]+[SPPGKPQGP PQQEGNKPQGPPPPGKPQ])/total protein (μg); c) having the subject initiate or resume a physical training program comprising activities of different intensity; d) measuring the concentration of a peptide selected from the group consisting of: 1) a peptide comprising the amino acid sequence PPGK-PQGPPPQGGNQPQGPPPPPGKPQ (SEQ ID NO:1), 2) a peptide comprising the amino acid sequence GNPQGPSPQGGNKPQGPPPPPGKPQ (SEQ ID NO:2), a 3) a peptide comprising the amino acid sequence SPPGK-PQGPPQQEGNKPQGPPPPGKPQ (SEQ ID NO:3), and 4) any combination thereof, in a saliva sample taken from the subject at one or more time points after (c), wherein the peptides of (d) are the same as the peptides of (a); e) identifying the subject's level of physical fatigue by calculating the ratio of the concentration of the peptide(s) measured in (d) to the total amount of protein in the sample, according to the ([PPGKPQGPPPQGGNQPQGPPPPPGKPQ]+ [GNPQGPSPQGGNKPQGPPPPPGKPQ]+[SPPGKPQGP-PQQEGNKPQGPPPPGKPQ])/total protein (μg); and f) guiding the subject's physical training program by modifying the intensity of subsequent activities using the latest of the subject's ratio(s), as calculated in (e), such that a decrease in the ratio relative to the previous ratio leads to a subsequent increase in the subject's training intensity, an increase in the ratio relative to the previous ratio leads to a subsequent decrease in the subject's training intensity, and a constant ratio relative to the previous ratio leads to a subsequent constant level in the subject's training intensity. In some embodiments, the physical training program can be a military training program.

The present invention also provides a method of evaluating the effect of a performance enhancing material and/or activity on the physical performance capability of a human subject, comprising: a) measuring the concentration of a peptide selected from the group consisting of: 1) a peptide comprising the amino acid sequence PPGKPQGPPPQGGNQPQGPPP-PPGKPQ (SEQ ID NO:1), 2) a peptide comprising the amino sequence GNPQGPSPQGGNKPQGPPPPPGKPQ (SEQ ID NO:2), a 3) a peptide comprising the amino acid sequence SPPGKPQGPPQQEGNKPQGPPPPGKPQ (SEQ ID NO:3), and 4) any combination thereof, in a saliva sample taken from the subject at a time point prior to exposing the subject to a performance enhancing material or performance enhancing activity, wherein the subject is an adult athlete or amateur athlete; b) identifying the subject's level of physical fatigue by calculating the ratio of the concentration of the peptide(s) measured in (a) to the total amount of protein in the sample, according to the equation: ([PPGKPQGPPPQGGN-QPQGPPPPPGKPQ]+[GNPQGPSPQGGNKPQGPPPP-PGKPQ]+[SPPGKPQGPPQQEGNKPQGPPPPGKPQ])/total protein (µg); c) exposing the subject to the performance enhancing material and/or performance enhancing activity; d) measuring the concentration of a peptide selected from the group consisting of: 1) a peptide comprising the amino acid

sequence PPGKPQGPPPQGGNQPQGPPPPPGKPQ (SEQ ID NO:1), 2) a peptide comprising the amino acid sequence GNPQGPSPQGGNKPQGPPPPPGKPQ (SEQ ID NO:2), a 3) a peptide comprising the amino acid sequence SPPGK-PQGPPQQEGNKPQGPPPPGKPQ (SEQ ID NO:3), and 4) 5 any combination thereof, in a saliva sample taken from the subject at one or more time points after (c), wherein the peptides of (d) are the same as the peptides of (a); e) identifying the subject's level of physical fatigue by calculating the ratio of the concentration of the peptide(s) measured in (d) to 10 the total amount of protein in the sample, according to the ([PPGKPQGPPPQGGNQPQGPPPPPGKPQ]+ equation: [GNPQGPSPQGGNKPQGPPPPPGKPQ]+[SPPGKPQGP-PQQEGNKPQGPPPPGKPQ])/total protein (μg); and f) evaluating the effect of the performance enhancing material 15 and/or activity on the physical performance capability of the subject by comparing the ratios of (b) and (e), wherein an increase in the ratio of (e) relative to the ratio of (b) is indicative of reduced physical performance capability of the subject, a decrease in the ratio of (e) relative to the ratio of (b) is 20 indicative of improved physical performance capability of the subject, and a constant ratio of (e) relative to the ratio of (b) is indicative of no change in physical performance capability of the subject.

DETAILED DESCRIPTION OF THE INVENTION

As used herein, "a" or "an" or "the" can mean one or more than one. For example, "a" cell can mean one cell or a plurality of cells.

Also as used herein, "and/or" refers to and encompasses any and/or all possible combinations of one or more of the associated listed items, as well as the lack of and and/or combinations when interpreted in the alternative ("or").

Furthermore, the term "about" as used herein when refering to a measurable value such as an amount of a compound or agent of this invention, dose, time, temperature, and the like, is meant to encompass variations of $\pm 20\%$, $\pm 10\%$, $\pm 5\%$, $\pm 1\%$, $\pm 0.5\%$, or even $\pm 0.1\%$ of the specified amount.

The present invention is explained in greater detail below. 40 This description is not intended to be a detailed catalog of all the different ways in which the invention may be implemented, or all the features that may be added to the instant invention. For example, features illustrated with respect to one embodiment may be incorporated into other embodiments, and features illustrated with respect to a particular embodiment may be deleted from that embodiment. In addition, numerous variations and additions to the various embodiments suggested herein will be apparent to those skilled in the art in light of the instant disclosure, which do not depart from the instant invention. Hence, the following specification is intended to illustrate some particular embodiments of the invention, and not to exhaustively specify all permutations, combinations and variations thereof.

The present invention is based on the unexpected discovery 55 of a modulation of biomarkers (e.g., modulation of peptide and/or protein levels) as measured in a biological sample from a subject that correlate with the subject's status with regard to having chronic fatigue syndrome, having an increased risk or likelihood of having or developing chronic 60 fatigue syndrome and/or having an altered (e.g., increased or decreased) level of fatigue over time.

Thus, in one embodiment, the present invention provides a method of identifying a subject as having chronic fatigue syndrome or having an increased likelihood of having or 65 developing chronic fatigue syndrome, comprising: a) measuring the amount of 1) a peptide comprising the amino acid

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sequence PPGKPQGPPPQGGNQPQGPPPPGKPQ, 2) a comprising the amino acid GNPQGPSPQGGNKPQGPPPPPGKPQ, and/or 3) a peptide comprising the amino acid sequence SPPGKPQGPPQQEG-NKPQGPPPPGKPQ, in any combination, in a biological sample from each subject in a population of subjects determined not to have chronic fatigue syndrome (e.g., non-CFS subjects); b) calculating the amount of the peptide(s) relative to the total amount of protein in each sample of (a) to determine a biomarker index for each subject of the population; c) establishing a threshold biomarker index from the biomarker indices determined in (b); d) measuring the amount of 1) a peptide comprising the amino acid sequence PPGKPQGPP-PQGGNQPQGPPPPPGKPQ, 2) a peptide comprising the amino acid sequence GNPQGPSPQGGNKPQGPPPP-PGKPQ, and/or 3) a peptide comprising the amino acid sequence SPPGKPQGPPQQEGNKPQGPPPPGKPQ, in any combination, in a biological sample from a test subject; and e) calculating the amount of the peptide(s) relative to the total amount of protein in the sample of (d) to determine a biomarker index for the test subject, wherein a biomarker index of the test subject that is higher than the threshold biomarker index of (c) identifies the subject as having chronic fatigue syndrome or having an increased likelihood of having or developing chronic fatigue syndrome.

In a further embodiment, the present invention provides a method of identifying a subject as having chronic fatigue syndrome or having an increased likelihood of having or developing chronic fatigue syndrome, comprising: a) measuring the amount of 1) human basic proline-rich protein 1 (PRB1), 2) human basic proline-rich protein 2 (PRB2), and/ or 3) human basic proline-rich protein 4 (PRB4), in any combination, in a biological sample from each subject in a population of subjects determined not to have chronic fatigue syndrome (e.g., non-CFS subjects); b) calculating the amount of the protein(s) relative to the total amount of protein in each sample of (a) to determine a biomarker index for each subject in the population; c) establishing a threshold biomarker index from the biomarker indices determined in (b); d) measuring the amount of 1) human basic proline-rich protein 1 (PRB1), 2) human basic proline-rich protein 2 (PRB2), and/or 3) human basic proline-rich protein 4 (PRB4), in any combination, in a biological sample from a test subject; and e) calculating the amount of the protein(s) relative to the total amount of protein in the sample of (d) to determine a biomarker index for the test subject, wherein a biomarker index of the test subject that is higher than the threshold biomarker index of (c) identifies the subject as having chronic fatigue syndrome or having an increased likelihood of having or developing chronic fatigue syndrome.

In an additional embodiment, the present invention provides a method of identifying a decrease over time in fatigue level of a subject, comprising: a) measuring, at a first time point, an amount of 1) a peptide comprising the amino acid sequence PPGKPQGPPPQGGNQPQGPPPPGKPQ, 2) a peptide comprising the amino acid sequence GNPQGPSPQGGNKPQGPPPPPGKPQ, and/or 3) a peptide comprising the amino acid sequence SPPGKPQGPPQQEG-NKPQGPPPPGKPQ, in any combination, in a first biological sample from the subject; b) calculating the amount of the peptide(s) of (a) relative to the total amount of protein in the first sample to determine a first biomarker index for the subject at the first time point; c) measuring, at a second or subsequent time point(s), an amount of 1) a peptide comprising the amino acid sequence PPGKPQGPPPQGGNQPQGPPP-PPGKPQ, 2) a peptide comprising the amino acid sequence GNPQGPSPQGGNKPQGPPPPPGKPQ, and/or 3) a peptide

comprising the amino acid sequence SPPGKPQGPPQQEG-NKPQGPPPGKPQ, in any combination, in a second or subsequent biological sample(s); and d) calculating the amount of the peptide(s) of (c) relative to the total amount of the protein in the second or subsequent sample(s) to determine a second or subsequent biomarker index for the subject at the second or subsequent time point(s), wherein a decrease in the second or subsequent biomarker index relative to the first biomarker index identifies a decrease over time in the fatigue level of the subject.

Furthermore, the present invention provides a method of identifying a subject as having chronic fatigue syndrome or having an increased likelihood of having or developing chronic fatigue syndrome, comprising: a) measuring an amount of 1) a peptide comprising the amino acid sequence 15 PPGKPQGPPPQGGNQPQGPPPPPGKPQ, 2) a peptide comprising the amino acid sequence GNPQGPSPQGGNK-PQGPPPPGKPQ, and 3) a peptide comprising the amino acid sequence SPPGKPQGPPQQEGNKPQGPPPPGKPQ, in any combination, in a biological sample from a test subject; 20 and b) calculating the amount of the peptide(s) relative to the total amount of protein in the sample of (a) to determine a biomarker index for the test subject, wherein a biomarker index of the test subject that is higher than a threshold biomarker index identifies the subject as having chronic fatigue 25 syndrome or having an increased likelihood of having or developing chronic fatigue syndrome.

The present invention also provides a method of identifying a subject as having chronic fatigue syndrome or having an increased likelihood of having or developing chronic fatigue 30 syndrome, comprising: a) measuring an amount of 1) human basic proline-rich protein 1 (PRB1), 2) human basic proline-rich protein 2 (PRB2), and 3) human basic proline-rich protein 4 (PRB4), in any combination, in a biological sample from a test subject; and b) calculating the amount of the 35 protein(s) relative to the total amount of protein in the sample of (a) to determine a biomarker index for the test subject, wherein a biomarker index of the test subject that is higher than a threshold biomarker index identifies the subject as having chronic fatigue syndrome or having an increased likelihood of having or developing chronic fatigue syndrome.

In an additional embodiment of this invention, a method is provided, of identifying a decrease over time in fatigue level of a subject, comprising: a) measuring, at a first time point, an amount of 1) human basic proline-rich protein 1 (PRB1), 2) 45 human basic proline-rich protein 2 (PRB2), and/or 3) human basic proline-rich protein 4 (PRB4), in any combination, in a first biological sample from the subject; b) calculating the amount of the protein(s) of (a) relative to the total amount of protein in the first sample to determine a first biomarker index 50 for the subject at the first time point; c) measuring, at a second or subsequent time point(s), an amount of 1) human basic proline-rich protein 1 (PRB1), 2) human basic proline-rich protein 2 (PRB2), and/or 3) human basic proline-rich protein 4 (PRB4) in a second or subsequent biological sample(s); and 55 d) calculating the amount of the protein(s) of (c) relative to the total amount of the protein in the second or subsequent sample(s) to determine a second or subsequent biomarker index for the subject at the second or subsequent time point(s), wherein a decrease in the second or subsequent 60 biomarker index relative to the first biomarker index identifies a decrease over time in the fatigue level of the subject.

In addition, the present invention provides a method of identifying an increase over time in fatigue level of a subject, comprising: a) measuring, at a first time point, an amount of 65 1) a peptide comprising the amino acid sequence PPGKPQG-PPPQGGNQPQGPPPPPGKPQ, 2) a peptide comprising the

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amino acid sequence GNPQGPSPQGGNKPQGPPPP-PGKPQ, and/or 3) a peptide comprising the amino acid sequence SPPGKPQGPPQQEGNKPQGPPPPGKPQ, in any combination, in a first biological sample from the subject; b) calculating the amount of the peptide(s) of (a) relative to the total amount of protein in the first sample to determine a first biomarker index for the subject at the first time point; c) measuring, at a second or subsequent time point(s), an amount of 1) a peptide comprising the amino acid sequence PPGKPQGPPPQGGNQPQGPPPPPGKPQ, 2) a peptide comprising the amino acid sequence GNPQGPSPQGGNK-PQGPPPPGKPQ, and/or 3) a peptide comprising the amino acid sequence SPPGKPQGPPQQEGNKPQGPPPPGKPQ, in any combination, in a second or subsequent biological sample(s); and d) calculating the amount of the peptide(s) of (c) relative to the total amount of the protein in the second or subsequent sample(s) to determine a second or subsequent biomarker index for the subject at the second or subsequent time point(s), wherein an increase in the second or subsequent biomarker index relative to the first biomarker index identifies an increase over time in the fatigue level of the subject.

In an additional embodiment of this invention, a method is provided, of identifying an increase over time in fatigue level of a subject, comprising: a) measuring, at a first time point, an amount of 1) human basic proline-rich protein 1 (PRB1), 2) human basic proline-rich protein 2 (PRB2), and/or 3) human basic proline-rich protein 4 (PRB4), in any combination, in a first biological sample from the subject; b) calculating the amount of the protein(s) of (a) relative to the total amount of protein in the first sample to determine a first biomarker index for the subject at the first time point; c) measuring, at a second or subsequent time point(s), an amount of 1) human basic proline-rich protein 1 (PRB1), 2) human basic proline-rich protein 2 (PRB2), and/or 3) human basic proline-rich protein 4 (PRB4) in a second or subsequent biological sample(s); and d) calculating the amount of the protein(s) of (c) relative to the total amount of the protein in the second or subsequent sample(s) to determine a second or subsequent biomarker index for the subject at the second or subsequent time point(s), wherein an increase in the second or subsequent biomarker index relative to the first biomarker index identifies an increase over time in the fatigue level of the subject.

Also provided herein is a method of identifying a subject as having chronic fatigue syndrome or having an increased likelihood of having or developing chronic fatigue syndrome, comprising: a) measuring the amount of two or more peptides selected from the group consisting of: 1) a peptide comprising the amino acid sequence PPGKPQGPPPQGGNQPQGPPP-PPGKPQ, 2) a peptide comprising the amino acid sequence GNPQGPSPQGGNKPQGPPPPPGKPQ, and 3) a peptide comprising the amino acid sequence SPPGKPQGPPQQEG-NKPQGPPPPGKPQ, in a biological sample from each subject in a population of subjects determined not to have chronic fatigue syndrome; b) calculating the amount of the peptides relative to the total amount of protein in each sample of (a) to determine a biomarker index for each subject of the population; c) establishing a threshold biomarker index from the biomarker indices determined in (b); d) measuring the amount of two or more peptides selected from the group consisting of: 1) a peptide comprising the amino acid sequence PPGKPQGPPPQGGNQPQGPPPPGKPQ, 2) a peptide comprising the amino acid GNPQGPSPQGGNKPQGPPPPPGKPQ, and 3) a peptide comprising the amino acid sequence SPPGKPQGPPQQEG-NKPQGPPPPGKPQ in a biological sample from a test subject (e.g., wherein the peptides of (d) are the same as peptides as measured in (a)); and e) calculating the amount of the

peptides relative to the total amount of protein in the sample of (d) to determine a biomarker index for the test subject, wherein a biomarker index of the test subject that is higher than the threshold biomarker index of (c) identifies the subject as having chronic fatigue syndrome or having an increased 5 likelihood of having or developing chronic fatigue syndrome.

Additionally provided herein is a method of identifying a subject as having chronic fatigue syndrome or having an increased likelihood of having or developing chronic fatigue syndrome, comprising: a) measuring the amount of two or 10 more proteins selected from the group consisting of 1) human basic proline-rich protein 1 (PRB1), 2) human basic prolinerich protein 2 (PRB2), and 3) human basic proline-rich protein 4 (PRB4), in a biological sample from each subject in a population of subjects determined not to have chronic fatigue 15 syndrome; b) calculating the amount of the proteins relative to the total amount of protein in each sample of (a) to determine a biomarker index for each subject in the population; c) establishing a threshold biomarker index from the biomarker indices determined in (b); d) measuring the amount of two or 20 more proteins selected from the group consisting of: 1) human basic proline-rich protein 1 (PRB1), 2) human basic proline-rich protein 2 (PRB2), and 3) human basic prolinerich protein 4 (PRB4), in a biological sample from a test subject, (e.g., wherein the proteins of (d) are the same proteins 25 as measured in (a)); and e) calculating the amount of the proteins relative to the total amount of protein in the sample of (d) to determine a biomarker index for the test subject, wherein a biomarker index of the test subject that is higher than the threshold biomarker index of (c) identifies the subject 30 as having chronic fatigue syndrome or having an increased likelihood of having or developing chronic fatigue syndrome.

The present invention also provides a method of identifying a subject as having chronic fatigue syndrome or having an increased likelihood of having or developing chronic fatigue 35 syndrome, comprising: a) measuring the amount of two or more peptides selected from the group consisting of: 1) a peptide comprising the amino acid sequence PPGKPQGPP-PQGGNQPQGPPPPPGKPQ, 2) a peptide comprising the amino acid sequence GNPQGPSPQGGNKPQGPPPP- 40 PGKPQ, and 3) a peptide comprising the amino acid sequence SPPGKPQGPPQQEGNKPQGPPPPGKPQ, in a biological sample from a test subject; and b) calculating the amount of the peptides relative to the total amount of protein in the sample of (a) to determine a biomarker index for the test 45 subject, wherein a biomarker index of the test subject that is higher than a threshold biomarker index identifies the subject as having chronic fatigue syndrome or having an increased likelihood of having or developing chronic fatigue syndrome.

Furthermore, the present invention provides a method of 50 identifying a subject as having chronic fatigue syndrome or having an increased likelihood of having or developing chronic fatigue syndrome, comprising: a) measuring the amount of two or more proteins selected from the group consisting of: 1) human basic proline-rich protein 1 (PRB1), 55 2) human basic proline-rich protein 2 (PRB2), and 3) human basic proline-rich protein 4 (PRB4), in a biological sample from a test subject; and b) calculating the amount of the proteins relative to the total amount of protein in the sample of (a) to determine a biomarker index for the test subject, 60 wherein a biomarker index of the test subject that is higher than a threshold biomarker index identifies the subject as having chronic fatigue syndrome or having an increased likelihood of having or developing chronic fatigue syndrome.

Furthermore, the present invention provides a method of 65 identifying a decrease over time in fatigue level of a subject, comprising: a) measuring, at a first time point, an amount of

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two or more peptides selected from the group consisting of: 1) a peptide comprising the amino acid sequence PPGKPQGP-PPQGGNQPQGPPPPGKPQ, 2) a peptide comprising the amino acid sequence GNPQGPSPQGGNKPQGPPPP-PGKPQ, and 3) a peptide comprising the amino acid sequence SPPGKPQGPPQQEGNKPQGPPPPGKPQ in a first biological sample from the subject; b) calculating the amount of the peptides of (a) relative to the total amount of protein in the first sample to determine a first biomarker index for the subject at the first time point; c) measuring, at a second or subsequent time point(s), an amount of 1) a peptide comprising the amino acid sequence PPGKPQGPPPQGGN-QPQGPPPPPGKPQ, 2) a peptide comprising the amino acid sequence GNPQGPSPQGGNKPQGPPPPPGKPQ, and 3) a peptide comprising the amino acid sequence SPPGKPQGP-PQQEGNKPQGPPPPGKPQ in a second or subsequent biological sample(s) (e.g., wherein the peptides measured in (c) are the same peptides as measured in (a)); and d) calculating the amount of the peptides of (c) relative to the total amount of the protein in the respective sample(s) to determine a second or subsequent biomarker index for the subject at the second or subsequent time point(s), wherein a decrease in the second or subsequent biomarker index relative to the first biomarker index identifies a decrease over time in the fatigue level of the subject.

In a further aspect of this invention, a method is provided, of identifying a decrease over time in fatigue level of a subject, comprising: a) measuring, at a first time point, an amount of two or more proteins selected from the group consisting of: 1) human basic proline-rich protein 1 (PRB1), 2) human basic proline-rich protein 2 (PRB2), and 3) human basic prolinerich protein 4 (PRB4) in a first biological sample from the subject; b) calculating the amount of the proteins of (a) relative to the total amount of protein in the first sample to determine a first biomarker index for the subject at the first time point; c) measuring, at a second or subsequent time point(s), an amount of two or more proteins selected from the group consisting of: 1) human basic proline-rich protein 1 (PRB1), 2) human basic proline-rich protein 2 (PRB2), and 3) human basic proline-rich protein 4 (PRB4) in a second or subsequent biological sample(s) (e.g., wherein the peptides measured in (c) are the same peptides as measured in (a); and d) calculating the amount of the proteins of (c) relative to the total amount of the protein in the second or subsequent sample(s) to determine a second or subsequent biomarker index for the subject at the second or subsequent time point(s), wherein a decrease in the second or subsequent biomarker index relative to the first biomarker index identifies a decrease over time in the fatigue level of the subject.

In yet further embodiments, the present invention provides a method of guiding a human subject's treatment for chronic fatigue syndrome (CFS), comprising: a) measuring the concentration of a peptide selected from the group consisting of 1) a peptide comprising the amino acid sequence PPGKPQG-PPPQGGNQPQGPPPPPGKPQ (SEQ ID NO:1), 2) a peptide comprising the amino acid sequence GNPQGPSPQGGNK-PQGPPPPPGKPQ (SEQ ID NO:2), 3) a peptide comprising the amino acid sequence SPPGKPQGPPQQEGNKPQGPP-PPGKPQ, (SEQ ID NO:3), and 4) any combination thereof, in a saliva sample taken from the subject; b) identifying the subject's level of chronic fatigue by calculating the ratio of the concentration of the peptide(s) measured in (a) to the total amount of protein in the sample, according to the equation: ([PPGKPQGPPPQGGNQPQGPPPPPGKPQ]+[GN-

PQGPSPQGGNKPQGPPPPPGKPQ]+[SPPGKPQGP-PQQEGNKPQGPPPPGKPQ])/total protein (μg); c) having the subject initiate or resume a treatment program for chronic

decreased chronic fatigue of the subject, and a constant ratio of (e) relative to the ratio of (b) is indicative of no change in chronic fatigue of the subject.

fatigue syndrome; d) measuring the concentration of a peptide selected from the group consisting of 1) a peptide comprising the amino acid sequence PPGKPQGPPPQGGN-QPQGPPPPGKPQ (SEQ ID NO:1), 2) a peptide comprising the amino acid sequence GNPOGPSPOGGNK-POGPPPPGKPO (SEO ID NO:2), 3) a peptide comprising the amino acid sequence SPPGKPOGPPOOEGNKPOGPP-PPGKPQ, (SEQ ID NO:3), and 4) any combination thereof, in a saliva sample taken from the subject at one or more time points after (c), wherein the peptides of (d) are the same as the peptides of (a); e) identifying the subject's level of chronic fatigue by calculating the ratio of the concentration of the peptide(s) measured in (d) to the total amount of protein in the sample of (d) according to the equation: ([PPGKPQGPP-PQGGNQPQGPPPPPGKPQ]+[GNPQGPSPQGGNKPQG-PPPPPGKPQ]+[SPPGKPQGPPQQEGNKPQGPPP-

PGKPQ])/total protein (µg); and f) guiding the subject's treatment for chronic fatigue syndrome by modifying the intensity of subsequent treatments using the latest of the 20 subject's ratio(s) as calculated in (e), such that an increase in chronic fatigue relative to the previous measurement of chronic fatigue leads to a subsequent increase in the subject's treatment intensity.

Also provided herein is a method of evaluating the effect of 25 a treatment material and/or activity on the chronic fatigue level of a human subject (e.g., a subject diagnosed as having, determined to have or suspected of having chronic fatigue syndrome (CFS), comprising: a) measuring the concentration of a peptide selected from the group consisting of 1) a peptide 30 comprising the amino acid sequence PPGKPQGPPPQGGN-QPQGPPPPPGKPQ (SEQ ID NO:1), 2) a peptide comprising the amino acid sequence GNPQGPSPQGGNKPQGPP-PPPGKPQ (SEQ ID NO:2), a 3) a peptide comprising the amino acid sequence SPPGKPQGPPQQEGNKPQGPPP- 35 PGKPQ (SEQ ID NO:3), and 4) any combination thereof, in a saliva sample taken from a subject at a time point prior to exposing the subject to a treatment material and/or activity; b) identifying the subject's level of chronic fatigue by calculating the ratio of the concentration of the peptide(s) measured in 40 (a) to the total amount of protein in the sample, according to equation: ([PPGKPQGPPPQGGNQPQGPPPP-PGKPQ]+[GNPQGPSPQGGNKPQGPPPPPGKPQ]+[SP-PGKPQGPPQQEGNKPQGPPPPGKPQ])/total (μg); c) exposing the subject to the treatment material and/or 45 performance enhancing activity; d) measuring the concentration of a peptide selected from the group consisting of: 1) a peptide comprising the amino acid sequence PPGKPQGPP-PQGGNQPQGPPPPPGKPQ (SEQ ID NO:1), 2) a peptide comprising the amino acid sequence GNPQGPSPQGGNK- 50 PQGPPPPGKPQ (SEQ ID NO:2), a 3) a peptide comprising the amino acid sequence SPPGKPQGPPQQEGNKPQGPP-PPGKPQ (SEQ ID NO:3), and 4) any combination thereof, in a saliva sample taken from the subject at one or more time points after (c), wherein the peptides of (d) are the same as the 55 peptides of (a); e) identifying the subject's level of chronic fatigue by calculating the ratio of the concentration of the peptide(s) measured in (d) to the total amount of protein in the sample, according to the equation: ([PPGKPQGPPPQGGN-QPQGPPPPPGKPQ]+[GNPQGPSPQGGNKPQGPPPP-PGKPQ]+[SPPGKPQGPPQQEGNKPQGPPPPGKPQ])/total protein (µg); and f) evaluating the effect of the treatment material and/or activity on the chronic fatigue status of the subject by comparing the ratios of (b) and (e), wherein an increase in the ratio of (e) relative to the ratio of (b) is indica- 65 tive of increased chronic fatigue of the subject, a decrease in the ratio of (e) relative to the ratio of (b) is indicative of

Additionally provided herein is a method of guiding a human subject's physical training activity, comprising: a) measuring the concentration of a peptide selected from the group consisting of: 1) a peptide comprising the amino acid sequence PPGKPQGPPPQGGNQPQGPPPPPGKPQ (SEQ ID NO:1), 2) a peptide comprising the amino acid sequence GNPOGPSPOGGNKPOGPPPPPGKPO (SEO ID NO:2), a 3) a peptide comprising the amino acid sequence SPPGK-PQGPPQQEGNKPQGPPPPGKPQ (SEQ ID NO:3), and 4) any combination thereof, in a saliva sample taken from the subject when the subject is in a rested state, wherein the subject is an adult athlete or an amateur athlete; b) identifying the subject's level of physical fatigue by calculating the ratio of the concentration of the peptide(s) measured in (a) to the total amount of protein in the sample, according to the equa-([PPGKPQGPPPQGGNQPQGPPPPPGKPQ]+[GN-POGPSPOGGNKPOGPPPPPGKPO1+[SPPGKPOGP-

PQQEGNKPQGPPPPGKPQ])/total protein (µg); c) having the subject initiate or resume a physical training program comprising activities of different intensity; d) measuring the concentration of a peptide selected from the group consisting of: 1) a peptide comprising the amino acid sequence PPGK-PQGPPPQGGNQPQGPPPPPGKPQ (SEQ ID NO:1), 2) a peptide comprising the amino acid sequence GNPQGPSPQGGNKPQGPPPPPGKPQ (SEQ ID NO:2), a 3) a peptide comprising the amino acid sequence SPPGK-PQGPPQQEGNKPQGPPPPGKPQ (SEQ ID NO:3), and 4) any combination thereof, in a saliva sample taken from the subject at one or more time points after (c), wherein the peptides of (d) are the same as the peptides of (a); e) identifying the subject's level of physical fatigue by calculating the ratio of the concentration of the peptide(s) measured in (d) to the total amount of protein in the sample, according to the ([PPGKPQGPPPQGGNQPQGPPPPPGKPQ]+ equation: [GNPQGPSPQGGNKPQGPPPPPGKPQ]+[SPPGKPQGP-PQQEGNKPQGPPPPGKPQ])/total protein (µg); and f) guiding the subject's physical training program by modifying the intensity of subsequent activities using the latest of the subject's ratio(s), as calculated in (e), such that a decrease in the ratio relative to the previous ratio leads to a subsequent increase in the subject's training intensity, an increase in the ratio relative to the previous ratio leads to a subsequent decrease in the subject's training intensity, and a constant ratio relative to the previous ratio leads to a subsequent constant level in the subject's training intensity. In some embodiments, the physical training program can be a military training program.

The present invention also provides a method of evaluating the effect of a performance enhancing material and/or activity on the physical performance capability of a human subject, comprising: a) measuring the concentration of a peptide selected from the group consisting of: 1) a peptide comprising the amino acid sequence PPGKPOGPPPOGGNOPQGPPP-PPGKPQ (SEQ ID NO:1), 2) a peptide comprising the amino acid sequence GNPQGPSPQGGNKPQGPPPPPGKPQ (SEQ ID NO:2), a 3) a peptide comprising the amino acid sequence SPPGKPQGPPQQEGNKPQGPPPPGKPQ (SEQ ID NO:3), and 4) any combination thereof, in a saliva sample taken from the subject at a time point prior to exposing the subject to a performance enhancing material or performance enhancing activity, wherein the subject is an adult athlete or amateur athlete; b) identifying the subject's level of physical fatigue by calculating the ratio of the concentration of the peptide(s) measured in (a) to the total amount of protein in the

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sample, according to the equation: ([PPGKPQGPPPQGGN-QPQGPPPPPGKPQ]+[GNPQGPSPQGGNKPQGPPPP-PGKPQ]+[SPPGKPQGPPQQEGNKPQGPPPPGKPQ])/total protein (µg); c) exposing the subject to the performance enhancing material and/or performance enhancing activity; 5 d) measuring the concentration of a peptide selected from the group consisting of: 1) a peptide comprising the amino acid sequence PPGKPQGPPPQGGNQPQGPPPPPGKPQ (SEQ ID NO:1), 2) a peptide comprising the amino acid sequence GNPQGPSPQGGNKPQGPPPPPGKPQ (SEQ ID NO:2), a 10 3) a peptide comprising the amino acid sequence SPPGK-PQGPPQQEGNKPQGPPPPGKPQ (SEQ ID NO:3), and 4) any combination thereof, in a saliva sample taken from the subject at one or more time points after (c), wherein the peptides of (d) are the same as the peptides of (a); e) identi- 15 fying the subject's level of physical fatigue by calculating the ratio of the concentration of the peptide(s) measured in (d) to the total amount of protein in the sample, according to the ([PPGKPQGPPPQGGNQPQGPPPPPGKPQ]+ [GNPOGPSPQGGNKPQGPPPPPGKPQ]+[SPPGKPQGP- 20 PQQEGNKPQGPPPPGKPQ])/total protein (μg); and f) evaluating the effect of the performance enhancing material and/or activity on the physical performance capability of the subject by comparing the ratios of (b) and (e), wherein an increase in the ratio of (e) relative to the ratio of (b) is indica- 25 tive of reduced physical performance capability of the subject, a decrease in the ratio of (e) relative to the ratio of (b) is indicative of improved physical performance capability of the subject, and a constant ratio of (e) relative to the ratio of (b) is indicative of no change in physical performance capability of 30

In methods wherein the level of more than one peptide or protein is measured for a subject, the biomarker index for the subject could be determined by any combination of the peptide or protein data. For example, the biomarker index might 35 be a simple sum of the different peptide or protein levels, e.g., simple addition of the peptide or protein levels and division of the sum by the total amount of protein in the sample. More complex combinations of peptide or protein data might also be used. For example, the biomarker index could be calcu- 40 lated as a weighted sum of the various peptides or proteins, whereby the measured peptide or protein levels are multiplied by independent weighting factors before the values are summed; it is possible that one of the weighting factors could be zero. Higher-order mathematical combinations of peptide 45 or protein levels might also be considered. For example, an equation for calculating the biomarker index might include a squared, cubed, or other higher-order term for one or more of the various peptides or proteins.

the subject.

biological entity that is produced by cells and/or by commensal flora, or substances that are produced by cells or commensal flora that might be then chemically modified by extracellular enzymes, free radicals produced by cells of the body and/or other naturally occurring processes and that is found, 55 for example, in the saliva, urine, blood, vaginal secretion, tears, feces, sputum, hair, nails, skin, wound fluid, nasal swab, lymph, perspiration, oral mucosa, vaginal mucosa, or the anus, or in serum or plasma obtained from blood. Thus, in the methods of this invention, the sample can be any biological 60 fluid or tissue that can be used in an assay of this invention, including but not limited to, serum, plasma, blood, saliva, semen, lymph, cerebrospinal fluid, prostatic fluid, urine, sputum, oral mucosa, nasal mucosa, duodenal fluid, gastric fluid, skin, endothelium, biopsy material from a salivary gland, 65 biopsy material of a parotid gland, biopsy material of other glands of the mouth, secretions of the salivary gland, secre32

tions of the parotid gland, secretions of other glands of the mouth, joint fluid, body cavity fluid, tear fluid, anal secretions; vaginal secretions, perspiration, whole cells, cell extracts, tissue, biopsy material, aspirates, exudates, slide preparations, fixed cells, tissue sections, etc.

In various embodiments of this invention, the biological sample can be prepared according to methods well known in the art and as described in the Examples section herein, to be a small molecular weight (SMW) sample. In particular embodiments of this invention, the biological sample is saliva.

In some embodiments, a biomarker of this invention can be, but is not limited to, a peptide or polypeptide comprising, consisting essentially of and/or consisting of the amino acid sequence PPGKPQGPPPQGGNQPQGPPPPPGKPQ, the amino acid sequence GNPQGPSPQGGNKPQGPPPP-PGKPQ, and/or the amino acid sequence SPPGKPQGP-PQQEGNKPQGPPPPGKPQ, singly or in any combination. In some embodiments, the biomarker of this invention can be, but is not limited to human basic proline-rich protein 1 (PRB1), human basic proline-rich protein 2 (PRB2), and/or 3) human basic proline-rich protein 4 (PRB4), the amino acid sequence and nucleotide sequence of each of which is known in the art, as exemplified in Table 2.

In further embodiments, a biomarker of this invention can be a peptide comprising, consisting essentially of or consisting of any fragment of human basic proline-rich protein 1 (PRB1), human basic proline-rich protein 2 (PRB2), and/or 3) human basic proline-rich protein 4 (PRB4). For example, the biomarker of this invention can be a peptide comprising, consisting essentially of or consisting of one or more 5 mers, 6 mers, 7 mers, 8 mers, 9 mers or 10 mers of the amino acid sequence of any of human basic proline-rich protein 1 (PRB1), human basic proline-rich protein 2 (PRB2), and/or 3) human basic proline-rich protein 4 (PRB4).

As a nonlimiting example, a biomarker of this invention can be a peptide comprising, consisting essentially of or consisting of at least about five amino acids (e.g., 5, 6, 7, 8, 9, 10, 11, 12, 13, 14) to at least about 15, 20, 25, 30, 35, 40, 45, 50, 50, 70, 80, 90, 100, 125, 150, 175 or 200 amino acids (e.g., 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 1120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, As used herein, "biomarker" can mean any chemical or 50 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, etc.), wherein the peptide comprises one or more (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20) of the 5 mer peptides listed in Table 1. As a nonlimiting example, a biomarker peptide of this invention can be 20 amino acids in length and can comprise two of the 5 mer peptides of Table 1, which can be contiguous as provided in the protein sequence from which the 5 mer peptides were derived. The remaining 10 amino acids can be on either side of each 5 mer, between each 5 mer or both. Furthermore, the remaining amino acids can be amino acids that are contiguous with the 5 mer peptides and/or noncontiguous with the 5 mer peptides (e.g., amino acids that are not present in the order provided in the protein sequence from which the 5 mer peptides were derived). The 5 mers of the PRB proteins

provided in Table 1 are exemplary, as the present invention also encompasses 6 mers, 7 mers, 8 mers, 9 mers, etc., of these PRB proteins as noted above, the amino acid sequence of any of which would be readily determined by one of skill in the

In further embodiments, a biomarker of this invention can comprise, consist essentially of or consist of any peptide or protein listed in Table 1, singly or in any combination. The biomarker of this invention can also comprise, consist essentially of or consist of any fragment of any peptide or protein 10 listed in Table 1.

Thus, in certain embodiments, the present invention is directed to a biomarker, which is a peptide or polypeptide (i.e., protein) as described herein and the present invention can employ or involve one or more of the peptides and pro- 15 teins set forth herein in any method and/or kit of this invention, singly and/or in any combination. In certain other embodiments, the present invention provides a nucleic acid encoding a biomarker of this invention and the methods of this invention can employ or involve one or more of the 20 nucleic acids of this invention in any method/and or kit of this invention.

A biomarker of this invention can be detected and/or quantified in a sample by a variety of methods well known in the art for detecting and/or quantifying substances in biological 25 samples. For example, for detecting and/or quantifying a biomarker that is a peptide or polypeptide, standard methods for detecting and/or quantifying peptides and/or polypeptides in sample can be employed. Nonlimiting examples of such methods include direct protein measurement, absorbance at 30 280 nm, absorbance at 205 nm, extinction coefficient assay, Lowry assay, biuret assay, Bradford assay, bicinchoninic acid assay (BCA), amido black assay, colloidal gold assay, immunoassay or other specific binding assay employing an antibody or ligand that specifically binds a peptide or polypep- 35 tide, protein separation assays such as electrophoresis, gas chromatography (GC), high performance liquid chromatography (HPLC), mass spectrometry (MS), etc., as are well known in the art.

Other methods of detection can include bioassays using 40 mammalian or bacterial cells wherein an output is proportional to the concentration of peptide in the sample solution, and solid phase methods wherein binding to a surface coated with a peptide recognizing molecule triggers an output electrical signal or change in optical property.

In some embodiments of this invention, peptides and/or proteins in a sample of this invention can be measured by BCA.

For detection and/or quantification of a nucleic acid encoding a biomarker of this invention, standard methods for detec- 50 tion and/or quantification of nucleic acids in a sample can be employed. Non-limiting examples include hybridization assays, amplification assays, sequencing protocols, etc., as are well known in the art.

of the amount of biomarker peptide or protein to the total amount of protein in the biological sample. For example, the biomarker index can be a value determined by calculating the relative amount of peptide in nmoles per µg of total protein (e.g., as determined by BCA) in the biological sample. The 60 term "threshold biomarker index" as used herein means the biomarker index calculated from the biomarker indices from a population of subjects that defines the threshold value for identifying a subject as having chronic fatigue syndrome or having an increased likelihood or risk of having or developing 65 chronic fatigue syndrome. The threshold biomarker index is calculated from the biomarker index of each subject in a

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population of subjects determined not to have chronic fatigue syndrome (e.g., non-CFS subjects). In some embodiments, a predetermined or previously established threshold biomarker index can be used in the methods described herein to identify a subject as having chronic fatigue syndrome or having an increased likelihood of having or developing chronic fatigue syndrome. Such a predetermined or previously established threshold biomarker index can be determined according to the teachings set forth herein (see, e.g., Example 3) as well as art-known teachings.

A subject of this invention is any animal in which identification of chronic fatigue syndrome, identification of increased likelihood or risk of having or developing chronic fatigue syndrome and/or identification of changes in fatigue level over time is needed or desired. In some embodiments, the subject is mammal and in particular embodiments the subject is a human. In other embodiments, the subject can be a horse, a dog or any other mammal about which the information obtained from the methods of this invention is needed or desired. In some embodiments, the subject can be diagnosed with chronic fatigue syndrome and/or have symptoms of chronic fatigue syndrome or other fatigue-related disorder and in some embodiments the subject can have no diagnosis or symptom(s) of chronic fatigue syndrome or any other fatigue-related disorder. A subject of this invention that has an "increased likelihood" or "increased risk" of having or developing chronic fatigue syndrome can be a subject having symptoms and/or signs of chronic fatigue syndrome or other fatigue associated disorder or such a subject can be a subject who is not having symptoms and/or signs of chronic fatigue syndrome or other fatigue-associated disorder. A subject of this invention can have an increased risk or increased likelihood of having or developing chronic fatigue syndrome due to environmental and/or genetic factors as would be known to one of skill in the art. By "increased likelihood" or "increased risk" of having or developing chronic fatigue syndrome it is meant that the increase is relative to a control (e.g., a subject whose biomarker index is at or below the threshold biomarker

In the methods of this invention employing measurements over time, the time intervals can be minutes, hours, days, weeks, months and/or years in any order and in any combination.

In the methods of this invention that recite a first time point and a second or subsequent time points or later time points, in some embodiments, the first time point can be prior to performance of an activity (e.g., physical, athletic, mental, training, normal activity of daily living, etc.) and the second or subsequent time points can be during and/or after performance of the activity. In some embodiments, the first time point can be during and/or after the performance of activity and the second or subsequent points can be at a later time following completion of the activity.

Physical activity and/or athletic activity as described The term "biomarker index" as used herein means the ratio 55 herein can be but is not limited to ultra-endurance exercise, a military operation, military training, running, walking, bicycling, weight lifting, swimming, a standardized physical test course including, for example, those used by the military, a triathlon, a biathlon, shooting of a rifle, shooting of a handgun, the aiming of computerized target equipment, staying awake, hiking, hiking while carrying a large burden on the back, physical activities of daily living and any combination thereof.

> As used herein, the term "ultra-endurance exercise" means a single continuous session of physical activity and/or athletic activity during which the subject performs said activity for a minimum of four hours with an average exertion equal to or

greater than 70% of ventalitory threshold, as described in Harger-Domitrovich et al., 2007, Medicine & Science in Sports & Exercise. For example, ten hours of continuous repetition of a one-hour exercise regimen consisting of 9 min. of upper-body ergometry, 19 min. of cycling, and 20 min. of treadmill walking with 1-min transition between modes, followed by a 10-min. rest and feeding period.

Military training is defined as the process of preparing military individuals and units to perform their assigned functions and missions, particularly to prepare for combat and wartime functions. "Covering every aspect of military activity, training is the principal occupation of military forces when not actually engaged in combat." (*Brassey's Encyclopedia of Land Forces and Warfare*, By Franklin D. Margiotta). Training may include for example physical tasks such as swimming, hiking when equipped with full military gear, running, moving stealthily, climbing, performing the aforementioned tasks under extreme environmental conditions such as high and low temperatures, high altitude or under conditions where flora and fauna pose significant hazard.

Military operations are defined for the purposes of this instant invention as the activities engaged in by soldiers, sailors and airmen during performance of duties during periods of war and peace. Military operations may include tasks related to engagement of enemy combatants. These tasks may 25 include, but are not limited to the following examples, pursuing the enemy on foot, flying unmanned drone aircraft, operating electronic equipment, manning guns in flight on an airplane, performing law enforcement duties and providing intelligence.

Normal activities of daily living, as defined by the National Cancer Institute, include eating, dressing, getting into or out of a bed or chair, taking a bath or shower, and using the toilet. Instrumental activities of daily living are activities related to independent living and include preparing meals, managing 35 money, shopping, doing housework, and using a telephone.

The terms "fatigue" and "fatigued state" as used herein mean weariness or exhaustion from labor, exercise, or stress, including loss of physical strength and bodily and mental capabilities.

The term "chronic fatigue" as used herein describes fatigue lasting about six or more consecutive months, which is not due or identified to be due to ongoing exertion or other medical conditions associated with fatigue.

"Chronic fatigue syndrome" or "CFS" as used herein 45 describes the condition or status of a subject (e.g., a patient) who meets at least one of the criteria set forth for example, in 1) the CDC definition (1994) and also called the Fukuda definition, 2) The Oxford criteria (1991), which includes CFS of unknown etiology and a subtype called post-infectious 50 fatigue syndrome (PIFS), 3) The 2003 Canadian Clinical working definition, which states that "[a] patient with ME/CFS will meet the criteria for fatigue, post-exertional malaise and/or fatigue, sleep dysfunction, and pain; have two or more neurological/cognitive manifestations and one or 55 more symptoms from two of the categories of autonomic, neuroendocrine, and immune manifestations; and the illness will persist for at least 6 months," 4) CFS/ME guideline for the National Health Service in England and Wales, produced in 2007 by the National Institute for Health and Clinical 60 Excellence (NICE), and 5) other criteria including gene expression markers, genetic profiles and/or biomarkers as are known in the art. See, e.g., "Chronic Fatigue Syndrome/ Myalgic Encephalomyelitis, A Primer for Clinical Practitioners" by the International Association for Chronic Fatigue 65 Syndrome/Myalgic Encephalomyelitis (IACFS/ME), 2012 Edition, the entire contents of which are incorporated by

reference herein. See also the following websites: www.iacf-sme.org and www.cdc.gov/cfs/general/index.html, the entire contents of each of which are incorporated by reference herein.

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A "treatment program for chronic fatigue syndrome" describes a physician-directed program including one or more of the treatments for Chronic Fatigue Syndrome recommended by the Centers for Disease Control and Prevention, International Association for Chronic Fatigue Syndrome/Myalgic Encephalomyelitis, and/or other recognized organizations with expertise in Chronic Fatigue Syndrome

As used herein, "increasing the intensity of a treatment program" means changing a subject's treatment program such that the supervising physician believes that the level or intensity of future treatment is greater than the previous level or intensity of treatment.

Nonlimiting examples of treatments for chronic fatigue syndrome (CFS) include 1) cognitive behavioral therapy, 2) graded exercise therapy as a form of physical therapy, 3) 20 pacing or energy management therapy, 4) pharmacotherapy including, e.g., Amitriptyline, Doxepin, Nortriptyline, Cyclobenzaprine, Trazodone, Gabapentin, Pregabalin, Promethazine, Diphenhydramine, Clonazepam, Orphenadrine, Ropinirole, pramipexole, Melatonin, Zolpidem, Zopiclone, Mirtazapine, Acetaminophen, Aspirin, Diclofenac, Gabapentin, Duloxetine, Codeine phosphate, oxycodone, hydrocodone, morphine, Tramadol, Modafinil, Armnodafanil, Methylphenindate, Dexamphetamine, Caffeine, Amphetamine salts Isoprinosine, anti-viral medications, rintatolimod, rituximab, antibiotics, anti-parasitics, dietary supplementation, vitamin D, vitamin B-12, B-complex vitamins, essential fatty acids, zinc and herbal remedies, light and radiation therapy, and any combination of these agents and therapies.

Also as used herein, the term "rested" or "rested state" or "non-fatigued state" means having sufficient rest from bodily and/or mental exertion, either before physical exercise and/or after recovery from fatigue.

"Perceived level of fatigue" as used herein means an individual's personal estimate or assessment of their fatigue and their ability to carry out tasks requiring a certain level of physical and/or cognitive performance

In addition, as used herein, "physical performance capability" means the capacity to accomplish a task which requires expenditure of a particular amount of energy. These tasks include, but are not limited to lifting objects, carrying objects, maintaining a certain pace of walking, running and/or cycling and maintaining a particular heart rate for a specified period. Capability represents a subject's potential for energy expenditure over a particular period of time.

The term "fitness" as used herein means good health or physical condition, especially as the result of exercise and proper nutrition.

In some embodiments of this invention, a subject of this invention is any animal in which identification of chronic fatigue syndrome, identification of increased likelihood or risk of having or developing chronic fatigue syndrome and/or identification of changes in fatigue level over time is needed or desired. In some embodiments, the subject is mammal and in particular embodiments the subject is a human. In other embodiments, the subject can be a horse, a dog or any other mammal about which the information obtained from the methods of this invention is needed or desired.

In some embodiments, the subject can be diagnosed with chronic fatigue syndrome and/or have symptoms of chronic fatigue syndrome or other fatigue-related disorder and in some embodiments the subject can have no diagnosis or

enhancing material include an herbal tea, warm milk, turkey, a large meal, an alcoholic beverage, a dietary supplement, a mud bath, a salt bath, an FDA-approved drug agent with a specific indication for treatment of a sleep disorder including, e.g., insomnia, narcolepsy, sleep apnea, depression, anxiety, and sleep disorder associated with shift work.

Furthermore, a "sleep enhancing activity" refers to actions taken by the subject to promote sleepiness and thereby sleep,

symptom(s) of chronic fatigue syndrome or any other fatiguerelated disorder. A subject of this invention that has an "increased likelihood" or "increased risk" of having or developing chronic fatigue syndrome can be a subject having symptoms and/or signs of chronic fatigue syndrome or other fatigue associated disorder or such a subject can be a subject who is not having symptoms and/or signs of chronic fatigue syndrome or other fatigue-associated disorder. A subject of this invention can have an increased risk or increased likelihood of having or developing chronic fatigue syndrome due 10 to environmental and/or genetic factors as would be known to one of skill in the art. By "increased likelihood" or "increased risk" of having or developing chronic fatigue syndrome it is meant that the increase is relative to a control (e.g., a subject whose biomarker index is at or below the threshold biomarker 15 index).

Furthermore, a "sleep enhancing activity" refers to actions taken by the subject to promote sleepiness and thereby sleep, and/or actions taken by others directed towards the subject to promote sleepiness and thereby sleep, and/or actions taken by an automated system directed towards the subject to promote sleepiness and thereby sleep. Nonlimiting examples of a sleep enhancing activity include reading while lying down, reading poetry, watching television, listening to music, exposure to electromagnetic fields, having sex, soaking in warm water, massage, counting sheep and/or other redundant mental activity.

In some embodiments, a population of study subjects of this invention includes healthy male and/or female volunteers less than the age of 42 that are in good physical condition and not suffering from known diseases and/or healthy young (less 20 than 25 years old) military members being screened for selection to Special Forces in the United States Military.

As used herein, "treatment of a sleep disorder" includes actions taken by a medical doctor or other medical practitioner on the subject to reduce symptoms associated with a sleep disorder or actions taken by a non-medical therapist or counselor on the subject to treat a sleep disorder or action taken by the spouse or friend or acquaintance of the subject to treat a sleep disorder or action taken by the subject to treat a sleep disorder. Nonlimiting examples of a treatment of a sleep disorder include taking an FDA approved agent and/or using a device for treatment of sleep disorder as prescribed by the subject's physician, psychological therapy delivered by a therapist to the subject to reduce anxiety and thereby mitigate sleep disorder, cognitive engagement by a spouse to improve marital relationship thereby reducing sleep disorder, a commitment and/or action on the part of the subject to work less thereby reducing sleep disorder. Enhancement of a treatment of a sleep disorder means increased intensity and/or frequency and/or dose and/or adding a new or additional treatment to an existing treatment regimen Reduction of a treatment of a sleep disorder means reduced intensity or frequency and/or dose and/or removing a treatment from an existing treatment regimen.

In some embodiments of this invention, a subject of this invention can be military personnel, a shift worker, a laborer, a truck driver, airline personnel, assembly line worker, a 25 patient at a sleep clinic, an amateur athlete, a professional athlete, a worker in the oil and/or gas industry, a train driver, an astronaut, a space traveler, a coal miner, an air traffic controller and any combination thereof. A subject of this invention can also be any subject engaged in training and/or 30 engaged in athletics, other employment or other activities outside of defined military duties. A subject of this invention may also be engaged in any work occupation as listed in the Standard Occupational Classification (SOC) System Manual, Version 2010, published by the US Department of Commerce 35 and/or Brassey's Encyclopedia of Land Forces and Warfare, By Franklin D. Margiotta (1996) and/or The International Classification of Sleep Disorders Revised (2001) (ISBN-10: 157488087X) and/or "Sleep Disorders and Sleep Deprivation: An Unmet Public Health Problem" Harvey R. Colten 40 and Bruce M. Altevogt, Editors, Committee on Sleep Medicine and Research, ISBN: 0-309-65727-X, 424 pages, (2006), the entire contents of each of which are incorporated by reference herein.

"Treatment of fatigue" as used herein means medical interventions and/or other physical and/or mental actions and/or changes in behavior to reduce fatigue. Nonlimiting examples of a treatment of fatigue include the use of medical interventions that may include drugs, surgery, and/or use of a approved medical device and/or approved medical therapy intended to reduce fatigue, the use of cognitive therapy and/or counseling to promote or elicit behaviors that reduce fatigue by reducing exposure to fatiguing activities and/or promoting activities that reduce fatigue. Specific non-limiting examples include taking a prescribed drug to promote restful sleep, thereby reducing fatigue, and/or engaging in fatigue mitigation counseling that leads, for example, to scheduling of more time for sleep.

As used herein, a "sleep schedule" refers to periods of time 45 allocated to sleep and taken at a specific time over some defined and recurring period. This can mean, as a non-limiting example, allocating a sleep period of 8 hours starting at 10 PM where the recurring period is 24 hours long where each recurring period starts at 6 AM. In another example one sleep 50 period is 2 hours and starts at 4 PM and a second period starts at 1AM and is 4 hours long where the recurring period is 24 hours long where each recurring period starts at 6 AM.

As used herein, the term "work load" refers to cognitive and/or physical tasks that are required and/or desired for living. This includes tasks that are performed at home and outside the home. This includes, for example tasks related to activities of daily living and/or tasks accomplished or performed at a subject's workplace.

Guiding a subject's sleep schedule can be done by modifying the duration of the sleep periods, the number of sleep 55 periods per day, and/or the time at which sleep periods are started. The recurring period may be determined, for example, by the subject's work schedule and/or any convenient recurring time pattern for example per day, week, month, etc.

As also used herein, "fitness for duty" means having the ability to perform cognitive and/or physical tasks associated with daily living and/or work and having the ability to perform these tasks within reasonable periods of time and at a certain level of quality.

Also as used herein, a "sleep enhancing material" refers to foods that are ingested, drugs that are administered by the oral route, intravenous route, transdermal route, intranasal route, via inhalation, by intra-ocular route, by vaginal route, and/or by rectal delivery route or other means, or complex mixtures that are delivered through a combination of routes in the form of a bath or immersion. Nonlimiting examples of a sleep

A "threshold ratio" describes a level of the ratio that is associated with a high likelihood of being able to perform a

specific physical and/or cognitive task and/or to be fit for duty or to be in a non-fatigued state.

To perform a physical activity and/or athletic activity at a sufficient level means the ability to perform a task at a level that is required for professional advancement, required to 5 pass a test, and/or required to complete a study. This also may include but is not limited to meeting individual personal physical and performance goals, satisfying job eligibility requirements, and/or meeting criteria required to continue working at a task, for example determining whether a person 10 is too fatigued to drive a truck.

In some embodiments, to be "sufficiently rested to carry out a work load" includes having the ability to perform a task at a level that is required for professional advancement, required to pass a test and/or meet a predetermined threshold 15 of performance and/or required to complete a study.

In some embodiments, a population of study subjects of this invention includes healthy male and/or female volunteers less than the age of 42 that are in good physical condition and not suffering from known diseases (e.g., determined not to 20 have chronic fatigue syndrome) and/or healthy young (less than 25 years old) military members being screened for selection to Special Forces in the United States Military.

To perform a physical activity and/or athletic activity at a sufficient level means the ability to perform a task at a level 25 that is required for professional advancement, required to pass a test, and/or required to complete a study. This also may include but is not limited to meeting individual personal physical and performance goals, satisfying job eligibility requirements, and/or meeting criteria required to continue 30 working at a task, for example determining whether a person is too fatigued to drive a truck.

In some embodiments, to be "sufficiently rested to carry out a work load" includes having the ability to perform a task at a level that is required for professional advancement, 35 required to pass a test and/or meet a predetermined threshold of performance and/or required to complete a study.

The term "chronic fatigue syndrome" as used herein describes an art-known syndrome, the signs and symptoms of which are described in the literature (see, e.g., Reeves et al. 40 "Prevalence of chronic fatigue syndrome in metropolitan, urban, and rural Georgia" Population Health Metrics 5:5 (2007), the entire contents of which are incorporated by reference herein). Common symptoms and signs of chronic fatigue syndrome include fatigue, loss of memory or concen- 45 tration, sore throat, enlarged lymph nodes in the neck and/or armpits, unexplained muscle pain, pain that moves from one joint to another without swelling or redness, headache of a new type, pattern or severity, unrefreshing sleep, and extreme exhaustion lasting more than 24 hours after physical or men- 50 tal exercise. To meet the diagnostic criteria of chronic fatigue syndrome, a subject typically must have unexplained, persistent fatigue for six months or more, along with at least four of the following signs and symptoms: loss of memory or concentration, sore throat, enlarged lymph nodes in the neck or 55 armpits, unexplained muscle pain, pain that moves from one joint to another without swelling or redness, headache of a new type, pattern or severity, unrefreshing sleep, and extreme exhaustion lasting more than 24 hours after physical or mental exercise.

The biomarkers and biomarker indices of this invention are correlated with chronic fatigue syndrome, fatigue, a fatigued state, an increase or decrease in fatigue, an increase or decrease in physical performance, a subject's perceived level of fatigue, recovery from a fatigued state, and/or an increased 65 or decreased likelihood of performing an activity at a sufficient level as described herein according to methods well

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known in the art and as disclosed in the Examples provided herein. In general, identifying such correlation involves conducting analyses that establish a statistically significant association and/or a statistically significant correlation between the presence of a biomarker or biomarker index or a combination of biomarkers or biomarker indices and a change in the subject (e.g., from rested to fatigued state, during and/or after performance of physical activity or other defined or standardized activity) as detected according to standard methods. An analysis that identifies a statistical association (e.g., a significant association) between the biomarker or biomarker index or between the combination of biomarkers or biomarker indices and the change in the subject establishes a correlation between the increase or decrease of the biomarker or biomarker index or combination of biomarkers or biomarker indices in a subject and the change being analyzed.

It would be well understood by one of skill in the art that the methods of the present invention can be carried out on multiple subjects and the data compiled to produce mean and median values that indicate fatigue, a fatigued state, an increase or decrease in fatigue, an increase or decrease in physical performance, a subject's perceived level of fatigue, recovery from a fatigued state, and/or an increased or decreased likelihood of performing a physical activity at a sufficient level according to this invention. It would also be understood that the statistical limits described by the data obtained from groups of subjects can be applied to individual subjects' response. Thus, it would be understood that in some embodiments of this invention, the methods of this invention can be carried out using a computer, wherein, for example, the data from multiple subjects are stored in a computer database and analyzed according to art-known methods of statistical and mathematical analysis to identify means, medians, trends, statistically significant changes, variances, etc.

Thus, in some embodiments, the methods of this invention can be carried out using a computer. Thus the present invention provides a computer-assisted method of identifying fatigue, a fatigued state, an increase or decrease in fatigue, an increase or decrease in physical performance, a subject's perceived level of fatigue, recovery from a fatigued state, and/or an increased or decreased likelihood of performing a physical activity at a sufficient level. The method involves the steps of (a) storing a database of biological data for a plurality of subjects, the biological data that is being stored including for each of said plurality of subjects: (i) a description of the status of the subject and/or physical/athletic activity performed by the subject, (ii) a description of any performance enhancing material and/or activity administered to, contacted with and/or implemented by the subject; (iii) a description of measurements according to art-known methods detecting a change in the status or performance in the subject; and (iv) a description of measurements of biomarkers or biomarker indices in the subject; and then (b) querying the database to determine the relationship between a change in the measurement of biomarkers or biomarker indices in the subject and change in performance or status of the subject. Such querying can be carried out prospectively or retrospectively on the database by any suitable means, but is generally done by statistical analysis in accordance with known techniques, as 60 described herein.

Compositions of the Invention

Further aspects of the present invention include an isolated peptide comprising, consisting essentially of, or consisting of the amino acid sequence of PPGKPQGPPPQGGNQPQGP-PPPGKPQ (SEQ ID NO:1), an isolated peptide comprising, consisting essentially of, or consisting of the amino acid sequence of GNPQGPSPQGGNKPQGPPPPPGKPQ (SEQ

ID NO:2), and/or an isolated peptide comprising, consisting essentially of or consisting of the amino acid sequence of SPPGKPQGPPQQEGNKPQGPPPPGKPQ (SEQ ID NO:3) and a composition comprising any of these isolated peptides, singly or in any combination in a pharmaceutically acceptable carrier.

Also provided herein is an isolated peptide comprising, consisting essentially of or consisting of about five amino acids to about 15, 20, 25, 30, 35, 40, 45, 50, 50, 70, 80, 90 or 100 amino acids (including any value between 5 and 100 not 10 explicitly recited herein), wherein the peptide comprises one or more (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20) of the 5 mer peptides listed in Table 1, as well as a composition comprising any of these isolated peptides, singly or in any combination in a pharmaceutically accept-15 able carrier.

"Pharmaceutically acceptable," as used herein, means a material that is not biologically or otherwise undesirable, i.e., the material may be administered to a subject along with the compositions of this invention, without causing substantial 20 deleterious biological effects or interacting in a deleterious manner with any of the other components of the composition in which it is contained. The material would naturally be selected to minimize any degradation of the active ingredient and to minimize any adverse side effects in the subject, as 25 would be well known to one of skill in the art (see, e.g., Remington's Pharmaceutical Science; latest edition). Exemplary pharmaceutically acceptable carriers for the compositions of this invention include, but are not limited to, sterile pyrogen-free water and sterile pyrogen-free physiological 30 saline solution, as well as other carriers suitable for injection into and/or delivery to a subject of this invention, particularly a human subject, as would be well known in the art.

In some embodiments of this invention, a biomarker peptide and/or protein of this invention can be used, in any 35 combination, as an antiviral, an antimicrobial, and/or an antifungal agent. Thus, the biomarker peptides and/or proteins of this invention can be used in methods of treating and/or preventing disorders such as disorders associated with fatigue (e.g., chronic fatigue syndrome), viral infection, disease associated with viral infection, microbial infection, disease associated with microbial infection, fungal infection, disease associated with fungal infection, and any combination thereof. Dosages, modes and regimens of administration for peptides and proteins as described herein would be determined by one of skill in the art according to art-known protocols (see, e.g., *Remington's Pharmaceutical Science*; latest edition).

In some embodiments, the present invention provides a biomarker protein or peptide of this invention, a nucleic acid 50 comprising a nucleotide sequence encoding a biomarker protein or peptide of this invention, a vector comprising said nucleic acid and a cell containing said vector. The biomarker, the nucleic acid, the vector and/or the cell can be present singly and/or in any combination in a composition comprising a pharmaceutically acceptable carrier.

In other embodiments of this invention, a nucleic acid having the nucleotide sequence or a substantially similar nucleotide sequence of the gene encoding a biomarker protein or peptide of this invention can be used as a probe in a 60 nucleic acid hybridization assay for the detection of nucleic acid encoding a biomarker protein or peptide in various tissues and/or body fluids of a subject of this invention. The probe can be used in any type of nucleic acid hybridization assay including Southern blots (Southern, 1975, *J Mol. Biol.* 65 98:508), Northern blots (Thomas et al., 1980, *Proc. Natl Acad. Sci.* U.S.A. 77:5201-05), colony blots (Grunstein et al.,

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1975, Proc. Natl Acad. Sci. U.S.A. 72:3961-65), slot blots, dot blots, etc. Stringency of hybridization can be varied depending on the requirements of the assay according to methods well known in the art. Assays for detecting nucleic acid encoding a protein in a cell, or the amount thereof, typically involve first contacting the cells or extracts of the cells containing nucleic acids therefrom with an oligonucleotide probe that specifically binds to nucleic acid encoding a protein or peptide as described herein (typically under conditions that permit access of the oligonucleotide to intracellular material), and then detecting the presence or absence of binding of the oligonucleotide probe thereto. Any suitable assay format can be employed (see, e.g., U.S. Pat. No. 4,358, 535; U.S. Pat. Nos. 4,302,204; 4,994,373; 4,486,539; 4,563, 419; and 4,868,104, the disclosures of each of which are incorporated herein by reference in their entireties).

As used herein, the terms peptide and polypeptide are used to describe a chain of amino acids, which correspond to those encoded by a nucleic acid. A peptide usually describes a chain of amino acids of from two to about 50 amino acids and polypeptide usually describes a chain of amino acids having more than about 50 amino acids. The term polypeptide can refer to a linear chain of amino acids or it can refer to a chain of amino acids, which have been processed and folded into a functional protein. It is understood, however, that 50 is an arbitrary number with regard to distinguishing peptides and polypeptides and the terms may be used interchangeably for a chain of amino acids around 50. The peptides and polypeptides of the present invention can be obtained by isolation and purification of the peptides and polypeptides from cells or body fluids or tissues where they are found naturally or by expression of a recombinant and/or synthetic nucleic acid encoding the peptide or polypeptide. The peptides and polypeptides of this invention can be obtained by chemical synthesis, by proteolytic cleavage of a polypeptide and/or by synthesis from nucleic acid encoding the peptide or polypeptide.

It is also understood that the peptides and polypeptides of this invention may also contain conservative substitutions where a naturally occurring amino acid is replaced by one having similar properties and which does not alter the function of the peptide or polypeptide. Such conservative substitutions are well known in the art. Thus, it is understood that, where desired, modifications and changes can be made in the nucleic acid sequence of the underlying gene(s) and/or amino acid sequence of the peptides and polypeptides of the present invention and still obtain a peptide or polypeptide having like or otherwise desirable characteristics. Such changes can occur in natural isolates or can be synthetically introduced using site-specific mutagenesis, the procedures for which, such as mismatch polymerase chain reaction (PCR), are well known in the art. One of skill in the art will also understand that polypeptides and nucleic acids that contain modified and/or synthetic amino acids and nucleotides, respectively (e.g., to increase the half-life and/or the therapeutic efficacy of the molecule), as are well known in the art, can be used in the methods of the invention.

"Nucleic acid" as used herein refers to single- or double-stranded molecules which may be DNA, comprised of the nucleotide bases A, T, C and G, or RNA, comprised of the bases A, U (substitutes for T), C, and G. The nucleic acid may represent a coding strand or its complement. Nucleic acids may be identical in sequence to a sequence that is naturally occurring or may include alternative codons that encode the same amino acid as that which is found in the naturally occurring sequence. Furthermore, nucleic acids may include codons that represent conservative substitutions of amino

acids as are well known in the art. The nucleic acids of this invention can also comprise any nucleotide analogs and/or derivatives as are well known in the art.

As used herein, the term "isolated nucleic acid" means a nucleic acid separated or substantially free from at least some of the other components of the naturally occurring organism. for example, the cell structural components commonly found associated with nucleic acids in a cellular environment and/or other nucleic acids. The isolation of nucleic acids can therefore be accomplished by well-known techniques such as cell lysis followed by phenol plus chloroform extraction, followed by ethanol precipitation of the nucleic acids. The nucleic acids of this invention can be isolated from cells according to methods well known in the art for isolating nucleic acids. Alternatively, the nucleic acids of the present invention can be synthesized according to standard protocols well described in the literature for synthesizing nucleic acids. Modifications to the nucleic acids of the invention are also contemplated, provided that the essential structure and func- 20 tion of the peptide or polypeptide encoded by the nucleic acid are maintained.

The present invention further provides a kit for detection and/or quantification of the biomarkers of this invention. In some embodiments, such a kit can comprise one or more 25 antibodies, ligands and/or aptamers, along with suitable buffers, wash solutions, dilution buffers, secondary antibodies, detection reagents, etc., for the detection of antigen/antibody complex formation, ligand/target complex formation and/or aptamer/target complex formation under various conditions. 30 In another embodiment, a kit of this invention can comprise a nucleic acid probe or primer that is complementary to a nucleotide sequence encoding a biomarker of this invention, along with suitable buffers, wash solutions, dilution buffers, detection reagents, etc. for the amplification of target nucleic acid and/or detection of nucleic acid hybridization under various conditions.

Thus, in some embodiments, the present invention provides a kit comprising an antibody that specifically reacts with a biomarker of this invention and reagents for detecting 40 antigen/antibody complex formation.

Further provided is a kit comprising an aptamer that specifically reacts with a biomarker of this invention and reagents for detecting aptamer/target molecule complex formation.

In addition, a kit is provided herein, comprising a nucleic 45 acid that hybridizes under high stringency conditions with a nucleic acid encoding a biomarker of this invention and reagents for detecting nucleic acid hybridization complex formation.

Screening Methods

In addition, the present invention provides a method of identifying a substance that binds a peptide or protein of this invention, comprising contacting the peptide or protein with a test compound under conditions whereby binding between the peptide or protein and the test compound can be detected; 55 and detecting binding between the peptide or protein and the test compound.

Further provided is a method of identifying a substance having the ability to inhibit or enhance the binding activity of a peptide or protein of this invention, comprising contacting 60 the substance with the peptide or protein under conditions whereby binding can occur and detecting a decrease or increase in the amount of binding in the presence of the substance as compared to a control amount of binding in the absence of the substance, thereby identifying a substance 65 having the ability to inhibit or enhance the binding activity of the peptide or protein.

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For the methods of this invention that employ the detection of binding, such assays are well known in the art and can employ, for example, an antibody, ligand and/or aptamer that binds a peptide of this invention either directly or indirectly.

Also provided herein is a method of identifying a compound that modulates the activity of a peptide or protein of this invention, comprising contacting the peptide or protein with a test compound under conditions whereby modulation of the activity of the peptide or protein can be detected. Because there is an association between fatigue and a change in the levels of the peptides and proteins of this invention, the peptides and proteins may serve a role in, for example, communicating a state of high energy demand to target organs, altering function of organs involved in mobilization of energy, modulating the activity of organs involved with the mobilization of energy stores including adipose tissue, the liver and muscle, modulating the activity of gastrointestinal mucosal leading to increased absorption of sugars, converting amino acids to sugars, or modifying the metabolic and enzymatic activity of commensal bacteria residing in the gastrointestinal tract leading to increased availability of sugars and free fatty acids that can be used to accomplish physical work by voluntary muscle, modulating the activity of the liver, pancreas, duodenum and other organs that secrete enzymes, emulsifiers and other substances that affect the processing of food, altering the distribution and targeting of sugars, lipids and proteins in the blood. These activities can be measured using in vitro cell-based assays with various output functions that can be used to determine activity, cell-free assays that measure association with specific receptors or important regulatory molecules, gene expression assays, and methods that involve measurement of functional outputs or alterations of metabolic production, fat mobilization and other phenomenon associated with fatigue or the ability to perform physical and cognitive tasks.

Additionally, the present invention provides a method of identifying immunomodulating activity in a peptide or protein of this invention, specifically by employing the peptide or protein in an assay for immunomodulating activity and detecting immunomodulating activity in the presence of the peptide or protein as compared to a control, thereby identifying immunomodulating activity in the peptide or protein. In this method, the assay for immunomodulating activity can be, but is not limited to, antibody production (or other assay to detect humoral immune response, T cell activation (or other assay to detect cellular immune response), nitric oxide production, interleukin 2 (IL-2) secretion and any combination thereof.

Furthermore, a method is provided herein of identifying antiviral, antimicrobial and/or antifungal activity in a peptide or protein of this invention, comprising employing the peptide or protein in an assay for antiviral antimicrobial and/or antifungal activity and detecting antiviral, antimicrobial and/or antifungal activity in the presence of the peptide or protein as compared to a control, thereby identifying antiviral, antimicrobial and/or antifungal activity in the peptide or protein. Protocols for identifying antiviral, antimicrobial and/or antifungal activity in a substance are well known in the art.

The term "antibody" as used herein, includes, but is not limited to a polypeptide encoded by an immunoglobulin gene or immunoglobulin genes, or a fragment thereof "Antibody" also includes, but is not limited to, a polypeptide encoded by an immunoglobulin gene or immunoglobulin genes, or a fragment thereof, which specifically binds to and recognizes the biomarkers of this invention.

The term "epitope" means an antigenic determinant that is specifically bound by an antibody. Epitopes usually consist of

surface groupings of molecules such as amino acids and/or sugar side chains and usually have specific three-dimensional structural characteristics, as well as specific charge characteristics.

The terms "specifically binds to" and "specifically reactive 5 with" refer to a binding reaction that is determinative of the presence of the antigen and antibody or aptamer and target in the presence of a heterogeneous population of proteins, nucleic acids and/or other biologics. Thus, under designated assay conditions, the specified antibodies and antigens and/or 10 aptamers and targets bind to one another and do not bind in a significant amount to other components present in a sample.

In some embodiments employing antibodies, a variety of immunoassay formats can be used to select antibodies specifically reactive with a particular antigen. For example, 15 solid-phase ELISA immunoassays are routinely used to select monoclonal antibodies specifically immunoreactive with an analyte. See Harlow and Lane (ANTIBODIES: A LABORATORY MANUAL, Cold Springs Harbor Publications, New York, (1988)) for a description of immunoassay 20 formats and conditions that can be used to determine specific immunoreactivity. Typically a specific or selective reaction will be at least twice background signal to noise and more typically more than 10 to 100 times greater than background.

An "immunologically reactive fragment" of a protein 25 refers to a portion of the protein or peptide that is immunologically reactive with a binding partner, e.g., an antibody, which is immunologically reactive with the protein itself.

Antibodies to biomarkers of this invention can be generated using methods that are well known in the art. Such 30 antibodies can include, but are not limited to, polyclonal, monoclonal, chimeric, humanized, fully human, single chain, Fab fragments, and/or fragments produced by an expression library, including e.g., phage display. (See, e.g., Paul, FUNDAMENTAL IMMUNOLOGY, 3rd Ed., 1993, Raven Press, 35 New York, for antibody structure and terminology.)

Antibody fragments that contain specific binding sites for a biomarker of this invention can also be generated. For example, such fragments include, but are not limited to, the F(ab')₂ fragments that can be produced by pepsin digestion of 40 the antibody molecule, and the Fab fragments that can be generated by reducing the disulfide bridges of the F(ab')₂ fragments. Alternatively, Fab expression libraries can be constructed to allow rapid and easy identification of monoclonal Fab fragments with the desired specificity (Huse et al., *Science* 254, 1275-1281 (1989)).

For the production of antibodies, various hosts including goats, rabbits, rats, mice, humans, and others, may be immunized by injection with a protein or any fragment or oligopeptide or conjugate thereof that has immunogenic properties. 50 Depending on the host species, various adjuvants can be used to increase the immunological response. Such adjuvants include, but are not limited to, Freund's complete and incomplete adjuvant, mineral gels such as aluminum hydroxide, and surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanin, and dinitrophenol. Examples of adjuvants used in humans include BCG (bacilli Calmette-Guerin) and *Corynebacterium parvum*.

Monoclonal antibodies can be prepared using any technique that provides for the production of antibody molecules by continuous cell lines in culture. These include, but are not limited to, the hybridoma technique, the human B-cell hybridoma technique, and the EBV-hybridoma technique (Kohler et al. (1975) *Nature* 256:495-497; Kozbor et al. (1985) *J.* 65 *Immunol. Methods* 81:31-42; Cote et al. (1983) *Proc. Natl. Acad. Sci.* 80:2026-2030; Cole et al. (1984) *Mol. Cell Biol.*

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62:109-120). Briefly, the procedure can be as follows: an animal is immunized with a protein or immunogenic fragment or oligopeptide or conjugate thereof. Lymphoid cells (e.g., splenic lymphocytes) are then obtained from the immunized animal and fused with immortalizing cells (e.g., myeloma or heteromyeloma) to produce hybrid cells. The hybrid cells are screened to identify those that produce the desired antibody.

Human hybridomas that secrete human antibody can be produced by the Kohler and Milstein technique and according to art-known protocols. Hybridoma production in rodents, especially mouse, is a very well established procedure and thus, stable murine hybridomas provide an unlimited source of antibody of select characteristics. As an alternative to human antibodies, the mouse antibodies can be converted to chimeric murine/human antibodies by genetic engineering techniques. See Oi et al. *Bio Techniques* 4(4):214-221 (1986); Sun et al. *Hybridoma* 5 (1986).

The monoclonal antibodies of this invention specific for biomarker epitopes of this invention can also be used to produce anti-idiotypic (paratope-specific) antibodies. (See e.g., McNamara et al., *Science* 220, 1325-26 (1984); Kennedy et al., *Science* 232:220 (1986).) These antibodies resemble the biomarker epitope and thus can be used as an antigen to stimulate an immune response against the biomarker.

In addition, techniques developed for the production of "chimeric antibodies," the splicing of mouse antibody genes to human antibody genes to obtain a molecule with appropriate antigen specificity and biological activity can be used (Morrison et al. *Proc. Natl. Acad. Sci.* 81:6851-6855 (1984); Neuberger et al., *Nature* 312:604-608 (1984); Takeda et al., *Nature* 314:452-454 (1985)). Alternatively, techniques described for the production of single chain antibodies can be adapted, using methods known in the art, to produce biomarker protein-specific single chain antibodies. Antibodies with related specificity, but of distinct idiotypic composition, can be generated by chain shuffling from random combinatorial immunoglobin libraries (Burton, *Proc. Natl. Acad. Sci.* 88:11120-3 (1991)).

Antibodies can also be produced by inducing in vivo production in the lymphocyte population or by screening immunoglobulin libraries or panels of highly specific binding reagents as described in the literature (Orlandi et al., *Proc. Natl. Acad. Sci.* 86:3833-3837 (1989)); Winter et al., *Nature* 349:293-299 (1991)).

Various immunoassays can be used to identify biomarkers of this invention. Such immunoassays typically involve the measurement of antigen/antibody complex formation between a biomarker protein or peptide and its specific antibody.

The immunoassays of the invention can be either competitive or noncompetitive and both types of assays are well-known and well-developed in the art. In competitive binding assays, antigen or antibody competes with a detectably labeled antigen or antibody for specific binding to a capture site bound to a solid surface. The concentration of labeled antigen or antibody bound to the capture agent is inversely proportional to the amount of free antigen or antibody present in the sample.

Noncompetitive assays of this invention can be sandwich assays, in which, for example, the antigen is bound between two antibodies. One of the antibodies is used as a capture agent and is bound to a solid surface. The other antibody is labeled and is used to measure or detect the resultant antigen/antibody complex by e.g., visual or instrument means. A number of combinations of antibody and labeled antibody can be used, as are well known in the art. In some embodi-

ments, the antigen/antibody complex can be detected by other proteins capable of specifically binding human immunoglobulin constant regions, such as protein A, protein L or protein G. These proteins are normal constituents of the cell walls of streptococcal bacteria. They exhibit a strong nonimmunogenic reactivity with immunoglobulin constant regions from a variety of species. (See, e.g., Kronval et al., *J. Immunol.*, 111:1401-1406 (1973); Akerstrom et al., *J. Immunol.*, 135: 2589-2542 (1985).)

In some embodiments, the non-competitive assays need 10 not be sandwich assays. For instance, the antibodies or antigens in the sample can be bound directly to the solid surface. The presence of antibodies or antigens in the sample can then be detected using labeled antigen or antibody, respectively.

In some embodiments, antibodies and/or proteins can be 15 conjugated or otherwise linked or connected (e.g., covalently or noncovalently) to a solid support (e.g., bead, plate, slide, dish, membrane or well) in accordance with known techniques. Antibodies can also be conjugated or otherwise linked or connected to detectable groups such as radiolabels (e.g., 20 ³⁵S, ¹²⁵I, ³²P, ¹³H, ¹⁴C, ¹³¹I), enzyme labels (e.g., horseradish peroxidase, alkaline phosphatase), gold beads, chemiluminescence labels, ligands (e.g., biotin) and/or fluorescence labels (e.g., fluorescein) in accordance with known techniques.

A variety of organic and inorganic polymers, both natural and synthetic can be used as the material for the solid surface. Nonlimiting examples of polymers include polyethylene, polypropylene, poly(4-methylbutene), polystyrene, polymethacrylate, poly(ethylene terephthalate), rayon, nylon, 30 poly(vinyl butyrate), polyvinylidene difluoride (PVDF), silicones, polyformaldehyde, cellulose, cellulose acetate, nitrocellulose, and the like. Other materials that can be used include, but are not limited to, include paper, glass, ceramic, metal, metalloids, semiconductive materials, cements and the 35 like. In addition, substances that form gels, such as proteins (e.g., gelatins), lipopolysaccharides, silicates, agarose and polyacrylamides can be used. Polymers that form several aqueous phases, such as dextrans, polyalkylene glycols or atoms) alkyl ammonium salts and the like are also suitable. Where the solid surface is porous, various pore sizes can be employed depending upon the nature of the system.

A variety of immunoassay systems can be used, including but not limited to, radio-immunoassays (RIA), enzyme- 45 linked immunosorbent assays (ELISA) assays, enzyme immunoassays (EIA), "sandwich" assays, gel diffusion precipitation reactions, immunodiffusion assays, agglutination assays, immunofluorescence assays, fluorescence activated cell sorting (FACS) assays, immunohistochemical assays, 50 protein A immunoassays, protein G immunoassays, protein L immunoassays, biotin/avidin assays, biotin/streptavidin assays, immunoelectrophoresis assays, precipitation/flocculation reactions, immunoblots (Western blot; dot/slot blot); immunodiffusion assays; liposome immunoassay, chemilu- 55 minescence assays, library screens, expression arrays, etc., immunoprecipitation, competitive binding assays and immunohistochemical staining. These and other assays are described, among other places, in Hampton et al. (Serological Methods, a Laboratory Manual, APS Press, St Paul, Minn. 60 (1990)) and Maddox et al. (J. Exp. Med. 158:1211-1216 (1993); the entire contents of which are incorporated herein by reference for teachings directed to immunoassays).

The methods of this invention can also be carried out using a variety of solid phase systems, such as described in U.S. Pat. 65 No. 5,879,881, as well as in a dry strip lateral flow system (e.g., a "dipstick" system), such as described, for example, in

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U.S. Patent Publication No. 20030073147, the entire contents of each of which are incorporated by reference herein.

In some embodiments, the biomarker of this invention can be detected and/or quantified in an assay employing an aptamer, a molecule that binds tightly to the biomarker in a manner similar to an antibody, a ligand or a small molecule. As used herein, the term "aptamer" includes any nucleic acid molecule or small peptide that specifically recognizes and binds a target molecule (e.g., a target peptide such as a biomarker of this invention). An "oligonucleotide-based aptamer" is defined as an aptamer made primarily, although not exclusively, from DNA and/or RNA bases. A "peptide-based aptamer" is defined as an aptamer made primarily, although not exclusively, from amino acids.

In some embodiments, an aptamer can be a small, usually stabilized, nucleic acid molecule that includes a binding domain for a target molecule (e.g., a biomarker of this invention). Oligonucleotide-based aptamers of this invention are oligonucleotides, or short (typically <100 bp) polymers of either DNA or RNA that have been selected from random pools based on their ability to bind nucleic acid, proteins, small organic compounds, and even entire organisms, usually with high affinity.

Oligonucleotide-based aptamers are typically developed to
bind particular ligands using a previously described selection
technique referred to as SELEX (Systematic Evolution of
Ligands by Exponential Enrichment). This technique allows
for selection of aptamers both in vivo and in vitro. Methods of
making aptamers are described in several publications, for
example, Ellington and Szostak, *Nature* 346:818 (1990),
Tuerk and Gold, *Science* 249:505 (1990), U.S. Pat. No. 5,582,
981, PCT Publication No. WO 00/20040, U.S. Pat. No. 5,270,
163, Lorsch and Szostak, *Biochemistry*, 33:973 (1994), Mannironi et al., *Biochemistry* 36:9726 (1997), Blind, *Proc. Nat'l.*35 Acad. Sci. USA 96:3606-3610 (1999), Huizenga and Szostak,
Biochemistry, 34:656-665 (1995), PCT Publication Nos. WO
99/54506, WO 99/27133, WO 97/42317 and U.S. Pat. No.
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aqueous phases, such as dextrans, polyalkylene glycols or surfactants, such as phospholipids, long chain (12-24 carbon atoms) alkyl ammonium salts and the like are also suitable. Where the solid surface is porous, various pore sizes can be employed depending upon the nature of the system.

A variety of immunoassay systems can be used, including but not limited to, radio-immunoassays (RIA), enzymelinked immunosorbent assays (ELISA) assays, enzyme immunoassays (EIA), "sandwich" assays, gel diffusion precipitation reactions, immunodiffusion assays, agglutination

Generally, in their most basic form, in vitro selection techniques for identifying oligonucleotide-based aptamers involve first preparing a large pool of oligonucleotides of the desired length that contain at least some central region that is randomized or mutagenized. For instance, a common oligonucleotide pool for aptamer selection might contain a region of 20-100 randomized nucleotides flanked on both ends by a relatively short (15-25 bp) region of nucleotides with defined sequence useful for the binding of PCR primers. The oligonucleotide pool is amplified using standard PCR techniques.

The original oligonucleotide pool is typically made of DNA bases. However, before the selection step, it can be converted to RNA bases using in vitro transcription methods well known in the art. During the selection step, the oligonucleotide library is allowed to interact with the target molecule, which is either free in solution or adhered to a physical surface such as a bead. In either case, the chemical environment of the interaction is typically controlled to simulate conditions anticipated for the final application of the invention, for example temperature, pH and osmolality matched to physiological conditions. When selection occurs in solution, capillary electrophoresis is used to separate bound from unbound oligonucleotides. For selection methods that use solid surfaces, bound and unbound oligonucleotide are separated by several rounds of washing of the surface. Bound oligonucleotide is isolated and amplified using standard PCR techniques. If the library was converted from DNA to RNA before selection, then reverse transcription must be used prior to PCR amplification. The amplified oligonucleotide

sequences are then put through another round of the same type of selection. Typically, the selection process requires a total of three to ten iterative rounds to produce a high-affinity aptamer. In the final step, the amplified DNA is cloned and sequenced using standard procedures to identify the sequence of the oligonucleotides that are capable of acting as aptamers for the target molecule. Once a sequence has been identified for a tightly binding oligonucleotide-based aptamer, the nucleotide-based aptamer may be further refined and optimized for binding affinity by performing additional rounds of selection starting from a pool of oligonucleotides containing controlled levels of randomized mutations of the original oligonucleotide sequence.

In further embodiments, an oligonucleotide-based aptamer can include at least one modified nucleotide base. The term "modified nucleotide base" encompasses nucleotides with a covalently modified base and/or sugar. For example, modified nucleotides include nucleotides having sugars that are covalently attached to low molecular weight organic groups other than a hydroxyl group at the 3' position and other than a phosphate group at the 5' position. Such modified nucleotides can also include 2' substituted sugars such as 2'-O-methyl; 2'-O-alkyl; 2'-O-alkyl; 2'-S-alkyl; 2'-S-allyl; 2'-fluoro; 2'-halo; or 2'-azido-ribose, carbocyclic sugar analogues, 25 a-anomeric sugars; epimeric sugars such as arabinose, xyloses or lyxoses, pyranose sugars, furanose sugars, and sedoheptulose.

Modified nucleotides of this invention can include but are not limited to, alkylated purines and/or pyrimidines; acylated purines and/or pyrimidines; and other heterocycles. These classes of pyrimidines and purines are known in the art and include, pseudoisocytosine; N4,N4-ethanocytosine; 8-hydroxy-N6-methyladenine; 4-acetylcytosine, 5-(carboxyhydroxylmethyl) uracil; 5-fluorouracil; 5-bromouracil; 5-carboxymethylaminomethyl-2-thiouracil;

5-carboxymethylaminomethyl uracil; dihydrouracil; inosine; N6-isopentyl-adenine; 1-methyladenine; 1-methylpseudouracil; 1-methylguanine; 2,2-dimethylguanine; 2-methylad- 40 enine; 2-methylguanine; 3-methylcytosine; 5-methylcy-N6-methyladenine; 7-methylguanine; 5-methylaminomethyl uracil; 5-methoxy amino methyl-2thiouracil; β-D-mannosylqueosine; 5-methoxycarbonylmethyluracil; 5-methoxyuracil; 2 methylthio-N6-isopenteny- 45 ladenine; uracil-5-oxyacetic acid methyl ester; psuedouracil; 2-thiocytosine: 5-methyl-2 thiouracil, 2-thiouracil: 4-thiouracil; 5-methyluracil; N-uracil-5-oxyacetic acid methylester; uracil 5-oxyacetic acid; queosine; 2-thiocytosine; 5-propyluracil; 5-propylcytosine; 5-ethyluracil; 5-ethylcytosine; 5-bu- 50 tyluracil; 5-pentyluracil; 5-pentylcytosine; and 2,6-diaminopurine; methylpseudouracil; 1-methylguanine; 1-methylcytosine.

Oligonucleotide-based aptamers of this invention can be synthesized from conventional phosphodiester linked nucleotides using standard solid or solution phase synthesis techniques that are known in the art. Linkages between nucleotides can use alternative linking molecules. For example, linking groups of the formula P(O)S, (thioate); P(S)S, (dithioate); P(O)NR'2; P(O)R'; P(O)OR6; CO; or CONR'2 wherein 60 R is H (or a salt) or alkyl (1-12C) and R6 is alkyl (1-9C) is joined to adjacent nucleotides through —O— or —S—.

In certain embodiments, the present invention can employ monoclonal or polyclonal nucleotide-based aptamers. A "monoclonal nucleotide-based aptamer" as used herein 65 includes a single aptamer with a known nucleotide sequence. A "polyclonal nucleotide-based aptamer" as used herein

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includes a population of aptamers with the same or different nucleotide sequences that all have an affinity for the same target molecule.

In other embodiments, an aptamer of this invention can be a recombinant protein or peptide that has been selected for specific binding to a target molecule according to methods known in the art (see, e.g., Hoppe-Seyler, Crnkovic-Mertens et al. 2004). The peptide-based aptamer can be a short peptide domain inserted into a supporting protein scaffold that enhances both specificity and affinity by conformationally constraining the peptide sequence (Colas, Cohen et al. 1996; Cohen, Colas et al. 1998; Buerger, Nagel-Wolfrum et al. 2003). In some embodiments of the present invention employing a peptide-based aptamer, the term "peptide-based aptamer" can be used to designate the peptide in the scaffold protein while the term "peptide" can refer to the inserted sequence.

In the methods of the present invention employing peptidebased aptamers, assays similar to the immunoassays described herein can be carried out to detect and/or quantify a biomarker of this invention, whereby a peptide-based aptamer is used in place of an antibody and an aptamer/target molecule complex, rather than an antibody/antigen complex is detected. The immunoassays described herein can also be adapted to employ an oligonucleotide-based aptamer in place of an antibody, for the detection of a nucleic acid/target molecule complex. In some embodiments, the immunoassays of this invention can also be modified to employ both aptamers and antibodies to detect and/or quantify a biomarker of this invention. Modification of any known immunoassay to accommodate the detection of binding of a nucleotide- or peptide-based aptamer to a target molecule would be well known to one of ordinary skill in the art.

As used herein, the term "signaling aptamer" includes aptamers with reporter molecules, such as a fluorescence dye, attached to the aptamer in such a way that upon conformational changes resulting from the interaction of the aptamer with a target molecule, the reporter molecule yields a differential signal, such as, for example, a change in fluorescence intensity. Alternatively, the amount of target molecule present may be quantified by the direct binding and retention of a fluorescently tagged aptamer on a solid surface or by the binding of a fluorescently tagged aptamer that recognizes the aptamer or antibody that binds specifically to the target molecule, i.e., secondary fluorescence assay. Examples of signaling aptamers can be found, for example, in U.S. Pat. No. 6,706,481, the entire contents of which are incorporated by reference herein for the disclosure of aptamers, methods of making aptamers and/or methods of using aptamers.

The present invention is more particularly described in the Examples set forth below, which are not intended to be limiting of the embodiments of this invention.

EXAMPLES

Example 1

Discovery of Salivary Biomarkers for Chronic Fatigue Syndrome Sample Collection and Liquid Chromatography-Mass Spectrometry (LC-MS) Analysis

Sample Collection

Saliva samples were collected using a cotton swab-based collection system (Salivette, Sarstedt, Newton, N.C.). Samples were stored at -80° C., shipped on dry ice and processed according to the manufacturer's instructions.

Protein Content

The level of protein in each saliva sample was quantified using the colorimetric bicinchoninic assay (BCA). Absorbance measurements (562 nm) and standard solutions were used to construct a calibration curve and linear regression was used to determine the final protein concentration for the unknown sample.

Size-Based Centrifugal Filtration

The supernatant was spun through a 50 kDa molecularweight cutoff (MWCO) filter (regenerated cellulose, Millipore, Billerica, Mass.) at 3,000 g for approximately 1 h at 22° C. The resulting filtrate was loaded into a 10 kDa MWCO filter (regenerated cellulose, Millipore) and spun for 1 h at 3,000 g at 22° C. The concentration of peptide and protein in the resulting filtrate was then determined using a commercially available kit for the BCA assay calibrated using bovine serum albumin standards. To remove salts and increase the concentration of peptides, ~100 µg of protein was passed through a commercially available peptide trap (Michrom, 20 Auburn, Calif.). The peptide concentration was again quantified using the BCA assay. The sample volume was then reduced using a heated centrifugal concentrator (Centrivap, Labconco, Kansas City, Mo.). Concentrated samples were labeled with a mass-specific variant of acetic anhydride, i.e., 25 acetic anhydride with either methyl protons or methyl deuterons (Yu et al., 2004). The labeling mixture consisted of a 1:250 dilution of mass-specific acetic anhydride prepared in ethanol with 50 mM triethylammonium bicarbonate. Samples were incubated for 1 h at 37° C. and then concentrated as described above. Finally, two aliquots (~2.5 µg each) from the same sample, labeled separately with light and heavy forms of acetic anhydride, were combined and injected onto the LC-MS system.

Ion-Trap Mass Spectrometry Detection

The various components of the processed saliva were separated using an ultra-high-pressure liquid chromatography (UPLC) system (Acquity, Waters, Milford, Mass.) with the outlet flowing directly into an ion-trap mass spectrometer (Bruker, Esquire 3000+, Billerica, Mass.). The UPLC was configured with a reversed-phase column (BEH300 C18, 1.7 µm particle, 2.1×100 mm, Waters) and the components were eluted from the column by varying the concentration of methanol in the running buffer linearly over a range from 10 45 to 35% at a flow rate of 0.3 ml/min. MS scans were collected at 2-5 Hz.

Analysis of LC-MS Data

A custom analysis program was written in LabVIEW (National Instruments, Austin, Tex.) to allow for the objective and automated identification of peak pairs within the data set separated by the expected mass-to-charge (m/z) differences appropriate for labeling with acetic anhydride, e.g., delta m/z of 3 for a singly charged ion that has been labeled with one acetate group, delta m/z of 6 for a singly charged ion that has been labeled with two acetate groups, etc. Because the overall number of ions identified in the CFS samples was lower than typical saliva samples examined previously, this analysis also included comparison of single ions, i.e., delta m/z of 0. Cluster analysis was used to identify peak pairs common to the 60 group at the beginning and end of the study.

High-Resolution MS Sequencing of Salivary Peptides

To determine the amino acid sequence of ions of interest, fractions (~1 min wide) of eluent near the elution time of the target ion were collected from LC injections of labeled saliva. 65 Five fractions were pooled for high-resolution mass spectrometric analysis (12T LTQ-FT Ultra, ProSight PC).

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Statistical Analysis

Statistical analysis was conducted using R (version 2.11.1) (Team 2010). For box plots, the horizontal line represents the median value while the boundaries of the rectangle indicate the range of the middle two quartiles. The whiskers indicate a distance 1.5× greater than the interquartile range from the nearest edge of the box. Open circles indicate points beyond the whiskers.

Results

Saliva samples were collected and analyzed as described above. The resulting LC-MS runs were analyzed with custom-written software, which enabled searches for clusters of mass-peak pairs within each group, i.e., searches for ions that were present in the LC-MS data for the majority of members of a particular group. Each LC-MS run was evaluated using the custom application and a list of detected mass pairs was written to a text file. In a typical LC-MS run, hundreds of thousands to millions of peaks were detected, of which several thousand were separated by one of the expected mass differences. The locations of these ion clusters were plotted with retention time serving as the x-coordinate and m/z serving as the y-coordinate.

From this plot of clusters, sites of potential biomarkers were identified, i.e., those sites for which a cluster appeared in one group, but not in the other. The coordinates of these sites were recorded and used to quantify the ion intensity at that location for each individual in the study. Data for three CFS-biomarker candidates are shown in FIGS. 1-3.

Quantifying Data Collected in an Ion Trap Mass Spectrometer

For the purposes of the study, the following approach for identifying biomarkers has been used. After the small-molecular-weight components of saliva were separated, the total protein concentration of the sample was estimated using a standard BCA assay. Using the estimated protein concentration, a total of 4 µg of protein was injected for each sample in an attempt to normalize the amount of material injected. High-Resolution Mass Spectrometry Data

Amino acid sequence data for three peptides were obtained using high-resolution mass spectrometry. The high-resolution analysis returned the following sequences for the three peptides using the single-letter amino acid notation: (1) GNPQGPSPQGGNKPQGPPPPGKPQ [bm_cfs_cand3], (2) PPGKPQGPPPQGGNQPQGPPPPGKPQ [bm_cfs_cand4], and (3) SPPGKPQGPPQQEGNKPQGPPPPGKPQ [sp_6]. The genes containing the sequences (1-3) are described below.

Genetic Information for Proteins Containing the Amino Acid Sequence of the Peptides

The Proline-rich Salivary Proteins (PRPs) constitute up to 70% of the soluble protein found in human saliva, and homologous proteins have been reported in non-human primates as well as in other animals, including rats, mice and hamsters. In humans, PRPs are the products of two gene families located on chromosome 12: (i) the HaeIII family, comprising two almost identical genes, PRH1 and PRH2, which code for acidic PRPs, and (ii) the BstN1 family, which includes four genes (PRB1, PRB2, PRB3 and PRB4) and codes for basic PRPs. With post-transcriptional and posttranslational processing, these six genes are responsible for at least thirteen different human protein products. In addition, a number of allelic forms, representing minor changes in amino acid composition, have also been identified for each of these genes. A variety of functions have been suggested for PRPs in saliva including protection against bacterial pathogens, regulation of calcium phosphate deposition, and most recently as a protective mechanism against dietary tannins and other phenolic compounds.

The biomarker with the amino acid sequence GNPQGPSPQGGNKPQGPPPPPGKPQ is derived from one of the PRB genes, PRB1, as a primary translation product containing 392 amino acids. These two PRB genes code for primary translation products of 392 amino acids (PRB1) and 416 amino acids (PRB2). Removal of the signal peptide produces Basic Salivary Proline-rich Protein 1 and Basic Salivary Proline-rich Protein 2, and further modifications yield several smaller products from each protein. Two of the final products of the PRB1 gene (Basic Salivary Proline-rich Protein 1 and Proline-rich Peptide II-2) contain amino acid sequence (1) [bm_cfs_cand3]. The method of release of the peptide into saliva is unclear. A detailed search of well-characterized proteases did not reveal any with enzymatic specificities that would generate this peptide fragment from the larger proteins.

The biomarker peptide with the amino acid sequence PPGKPQGPPPQGGNQPQGPPPPPGKPQ derives from one, or perhaps both, of a pair of the basic proline-rich protein 20 genes, PRB1 and PRB2, which are closely linked to the PRH genes. These two genes code for primary translation products of 392 amino acids (PRB1) and 416 amino acids (PRB2). Removal of the signal peptide produces Basic Salivary Proline-rich Protein 1 and Basic Salivary Proline-rich Protein 2, and further modifications yield several smaller products from each protein. Three of the final products of the PRB1 gene (Basic Salivary Proline-rich Protein 1, Proline-rich Peptide II-2 and Basic Peptide IB-6) and three from the PRB2 gene (Basic Salivary Proline-rich Protein 2, Basic Proline-rich 30 Peptide IB-7 and Basic Proline-rich Peptide IB-8c) contain amino acid sequence (2) [bm_cfs_cand4]. As with sequence (1), it is as yet unclear whether only one or both of the PRB1 and PRB2 genes is the source of amino acid sequence (2).

The biomarker peptide with the amino acid sequence 35 SPPGKPQGPPQQEGNKPQGPPPPGKPQ derives from one of the basic proline-rich protein genes, PRB4. The gene codes for a primary translation products of 247 amino acids. Removal of the signal peptide produces Basic Salivary Proline-rich Protein 4, and further modifications yield several 40 smaller products from each protein. Only one of the final products of the PRB4 gene (Basic Salivary Proline-rich Protein 4) contains amino acid sequence (3) [sp_6].

Example 2

Measurement of Biomarker(s)

- 1. Collect a sample of saliva from the test subject.
 - a. Collect saliva by having the subject spit directly into a 50 collection vial or tube.
 - b. Collect saliva using a matrix-mediated approach, such as the commercially available Salivette system developed by Salimetrics.
- 2. Prepare sample for injection into liquid chromatogra- 55 phy-mass spectrometry (LC-MS) system.
 - a. For saliva collected as in (la):
 - i. Spin saliva at 4 k g for 45 min at 4° C.
 - Determine protein concentration using bicinchoninic acid (BCA) assay with bovine serum albumin 60 (BSA) as standard.
 - Process saliva through molecular-weight-cutoff filters using 50 kDa and 10 kDa filters, sequentially.
 - iv. Pass final supernatant through peptide trap (Michrom, C8).
 - Determine protein concentration using BCA assay with BSA as standard.

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- vi. Dry the sample in a heated chamber with reduced pressure (Centrivap).
- vii. Reconstitute the sample in water with 0.1% acetic acid at a concentration of 0.1 µg of protein/µl.
- viii. Inject a sample containing 4 μg of protein, as determined by BCA assay, into LC-MS system.
- b. For saliva collected as in (1b):
 - Extract raw saliva from matrix according to manufacturer's instructions.
 - ii. Follow steps (2. a.ii-vii)
- 3. Run LC-MS analysis using a linear gradient of acidified (0.1%) water and methanol (95% to 65% water) to elute compounds from a reversed-phase column.
- 4. Measure the height of the peak for peptides of interest, as described herein.
- 5. Inject into the LC-MS system standard peptide solutions in at least three different concentrations using concentration values falling within the normal range of each peptide for 4 µg human saliva.
- Create a calibration curve for the peptide standards by plotting peak height vs. concentration.
- 7. For each peptide of interest, use the standard curve results in (6) and divide by 4 μg to determine the relative amount of peptide per μg of total salivary protein, i.e., the biomarker index for each peptide.

Example 3

Determining a Threshold Ratio

- 1. Using the measurement method described in Example 2, measure the level of peptide in each sample collected from a group of subjects (e.g., a population) determined not to have chronic fatigue syndrome (non-CFS subjects). Such a determination can be made for example as set forth in Reeves et al. ("Prevalence of chronic fatigue syndrome in metropolitan, urban, and rural Georgia" *Population Health Metrics* 5:5 (2007), the entire contents of which are incorporated by reference herein).
- 2. Determine the median value of the peptide for the population of non-CFS subjects.
- 3. Determine the standard deviation of the peptide for the population of non-CFS subjects.
- 4. Set the threshold ratio for identifying a subject as having chronic fatigue syndrome or having an increased likelihood of having or developing chronic fatigue syndrome equal to the following: Two times the standard deviation as determined in (3) plus the median as determined in (2).

Example 4

Novel Salivary Biomarker Associated with Chronic Fatigue Syndrome

Background.

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At present, diagnosis of chronic fatigue syndrome (CFS) requires a lengthy and expensive period of clinical examination to rule out all other causes of fatigue. Thus, many patients do not receive timely treatment or are misdiagnosed. Definitive rapid diagnosis is needed to improve the clinical outcomes for patients suffering from CFS.

Methodology/Principal Findings.

Saliva samples were obtained during a survey of the population of the state of Georgia (United States). The goal of this study was to determine incidence of undiagnosed CFS in the general population. Samples of saliva were maintained as a

frozen archive until time of analysis. From the archive, samples from 46 subjects with CFS and 45 samples from normal controls were selected for further analysis. The ionizable components below 5 kDa were identified using liquid chromatography-mass spectrometry (LC-MS). Patterns of 5 ion intensity as functions of retention time and mass-tocharge ratio were compared to identify significant differences between the CFS and control groups. A single biomarker candidate for CFS was identified. This biomarker candidate was approximately three times more abundant in saliva from 10 CFS subjects than in saliva from control subjects (p<0.001, Wilcoxon's signed rank). The sensitivity and specificity of the biomarker candidate with respect to correctly identifying CFS are 88 and 91%, respectively. The Receiver Operating Characteristic (ROC) Area-Under-the-Curve (AUC) is 0.935 (95% CI 0.864 to 0.976). De novo sequencing by high-resolution MS revealed that the biomarker candidate was a peptide of molecular weight 2,633 Da. The amino acid sequence of the identified peptide is found within the sequence of the saliva-specific 42 kDa basic Proline-Rich-Protein, PRB4.

Conclusions/Significance.

A salivary peptide identified as a candidate biomarker for CFS may facilitate rapid diagnosis of CFS.

Chronic fatigue syndrome is an orphan disease wrapped in mystery. The etiology of the disease is not clear nor is its 25 diagnosis and prognosis. Even the Fukuda definition, so frequently cited, describes CFS as unexplained fatigue [1]. The absence of clear etiological contributions is highlighted by the variety of labels for the condition including immune dysfunction syndrome, neuroendocrine immune dysfunction 30 syndrome, allergic encephalomyelitis, post viral syndrome and neurasthenia, among others. The self-reported symptoms consistent with CFS include intense fatigue with a duration greater than six months, which is not relieved by rest and causes tiredness that impairs performance of daily activities. 35 CFS is associated with a wide spectrum of symptoms including pain, headaches, cognitive disorders, sleep disorders, anxiety, depression and fatigue exacerbated by exercise. The search for effective treatments has been hampered by the lack of comprehension of the molecular and cellular basis for the 40 development and progression of CFS.

The intense fatigue in CFS has been a cause of confusion with several other chronic conditions such as fibromyalgia, irritable bowel syndrome, and temporomandibular joint syndrome. A lengthy clinical evaluation, including a complete 45 and detailed medical history, should be conducted to rule out other causes of fatigue and to characterize fatigue's form. time of onset, durations, triggering factors, relationship with rest and physical activities. The lack of objective criteria, specific signs and/or tests for the diagnosis of disease has led 50 to an underestimation of CFS prevalence [2]. Although some have estimated that more than 800,000 people suffer from CFS in the US [3], causing a loss of \$9 billion annually just in earnings and productivity [4], CDC has projected that only 9-16% of individuals with CFS had been diagnosed [5]. Even 55 when detected, the average time from the beginning of the symptoms to the diagnosis of the syndrome is around 5 years [6]. Because the fatigue associated with CFS is so severe, patients with CFS are 4 times more likely to forgo needed healthcare than non-fatigued subjects [7], worsening the 60 overall health outlook beyond the immediate impact of the disease itself.

Specific salivary peptides can be used to determine the physical fatigue status of athletes and adults during exercise [10]. Specifically the ratio of two endogenous peptides 65 declined by approximately 1,000 fold from a rested state to a physically fatigued state over a period of several hours. Fol-

lowing rest, the peptide levels recovered. Both peptides are derived from a family of saliva specific proteins call Proline Rich Proteins (PRPs). These proteins and their associated peptides are only found in the saliva. In a separate study, the ratio of these peptides, termed the Fatigue Biomarker Index or FBI, was used to investigate physical fatigue levels of candidates for US military Special Forces [11]. These studies showed that a single measurement of the FBI made prior to the start of a rigorous selection process lasting 12 weeks was one of only four variables needed to predict who would ultimately pass and who would fail. In general, those candidates who failed, for reasons related to poor physical performance, had lower FBI levels, and thus higher levels of baseline fatigue than those individuals that passed. This suggests that levels of the FBI may indicate a physiological state of fatigue that is persistent and ultimately affects performance capability over a relatively long period of time. Taken together these findings suggested that saliva may provide an objective means of evaluating chronic fatigue.

In the current study, a comparative proteomic analysis directed at the low molecular weight, <5 kD, fraction of saliva was conducted. The goal of the study was to identify differences between CFS and control subjects. The results demonstrate that there is at least one peptide, derived from the family PRPs, which is significantly elevated in CFS patients relative to controls. This peptide offers promise as an objective diagnostic laboratory test for CFS.

Study Subjects

Study saliva samples were obtained from a large crosssectional population based study of CFS and chronic unwellness in Georgia, investigating the prevalence of CFS between September 2004 and July 2005 conducted by the Centers for Disease Control and Prevention[7]. Briefly, 10,837 households with 21,165 members were contacted initially by telephone interview. At the end of screening and selection a total of 112 participants met established clinical criteria for CFS using the criteria established by the 1994 international research case definition [1] using validated test instruments as specified by the International CFS Study Group [12] and CDC standards [13]. A total of 147 subjects identified as non-fatigued were identified during the Georgia study. The control subjects were clinically evaluated and saliva samples were obtained. Saliva samples were collected from these subjects and controls for the purpose of determining salivary cortisol levels [14]. In all cases, the sample obtained at 8 AM was used for the purpose of biomarker discovery. A total of 46 and 45 CFS and control saliva samples, respectively, were obtained for the purpose of biomarker discovery (FIG. 4). Samples were stored at -80° C. and shipped on dry ice until thawed for processing.

Processing of Saliva

Saliva samples were thawed at room temperature and spun for 10 minutes to remove particulates. The resulting supernatant was filtered sequentially through 50 kDa and 10 kDa molecular-weight cutoff filters (regenerated cellulose, Millipore, Billerica, Mass.) to produce a low molecular weight fraction of saliva. Approximately 100 ug of peptide, as determined by BCA, was desalted using a C-8 column designed for this purpose (Michrom, Auburn, Calif.).

Ion-Trap Mass Spectrometry to Identify Putative Biomarkers of CFS

Ion-trap mass spectrometers are capable of detecting components between a m/z of 150 to 2,000 with a precision of 0.1 m/z. The processed low-molecular weight saliva fraction was introduced onto an ultra-high-pressure liquid chromatography (UPLC) system (Acquity, Waters, Milford, Mass.) with the outlet flowing directly into an ion-trap mass spectrometer

(Bruker, Esquire 3000+, Billerica, Mass.). The UPLC was configured with a reversed-phase column (BEH300 C18, 1.7 µm particle, 2.1×100 mm, Waters) and the components were eluted from the column by varying the concentration of methanol in the running buffer linearly over a range from 10 5 to 35% at a flow rate of 0.3 ml/min. MS scans were collected at 2-5 Hz. An analysis program (PeakQuest, Hyperion Biotechnology, San Antonio, Tex.) was used to identify putative biomarkers. PeakQuest enables identification of changes in the relative abundance of peptides. In this case, PeakQuest identifies component peptides that vary significantly in relative abundance compared to normal controls.

Definitive Chemical Identification of CFS Biomarkers

All putative biomarkers were found in a normal healthy pool of standard saliva (Hyperion Biotechnology Inc., San 15 Antonio, Tex.). Portions of normal pool saliva were processed in the manner described and fractions 1 minute wide corresponding to the elution time were collected. Fractions were combined and concentrated and evaluated using high-resolution mass spectrometry (HRMS) (12T LTQ-FT Ultra, Pro 20 Sight PC). HRMS provides a very precise estimation of peptides and peptide fragment mass, e.g., m/z±0.00001. During HRMS, peptide ions are fragmented by electron bombardment leading to specific disruption of peptide bonds. The disruption leads to the production of smaller peptides and 25 individual amino acids obtained from the parent peptide. The mass of these resulting fragments is determined with great precision. The estimated masses acquired in this manner are then compared to theoretical masses that are found on extensive tables. The theoretical composition of the fragments 30 enables a reconstruction of the parent peptide. This process is known as de novo sequencing. The de novo sequence determined in this manner can be confirmed by synthesizing the peptide called for from the de novo sequence. If the synthesized peptide demonstrates similar retention time and m/z on 35 the system used for discovery (ion-trap instrument), the identity of the biomarker is confirmed. The de novo sequence data was used to direct synthesis of biomarker peptides. Milligram quantities of biomarkers were synthesized (Anaspec, Fremont, Calif.). The elution times and MS/MS fragmentation 40 patterns of the synthesized peptides were observed and compared to similar data obtained from processed saliva. Statistical Analysis

Statistical analysis was conducted using SYSTAT (SPSS Inc., Chicago, Ill.). The receiver operator characteristic 45 (ROC) curve was calculated and analyzed using MedCalc ver. 12.2.1, (MedCalc Software, Mariakerke, Belgium). The standard error associated with ROC associated Area Under the Curve (AUC) was calculated using the method of DeLong [15]. Comparison between control and CFS was made by 50 t-tests. Comparisons of distribution of subjects to control and CFS groups according to lifestyle, health factors and demographic factors were made using the Chi-square statistic.

A biomarker candidate for CFS was identified. Specifically, levels of the biomarker candidate were higher in CFS subjects than control as shown in FIG. 5. Setting a threshold value to 28,840 intensity units leads to a test with sensitivity of 91% and specificity of 88%. With regard to CFS, it is desirable to provide the highest possible confidence with regard to diagnosis. To increase confidence in positive diagnosis, a measured value above 35,000 provides 100% confidence of a diagnosis of CFS. However, at this level, 8 of 46 patients that are identified as having CFS based on clinical signs are incorrectly determined to be normal.

Subsequently, de novo sequencing of the putative biomar-65 ker using a high-resolution mass spectrometer led to chemical identification of the biomarker candidate, a peptide with the

amino acid sequence SPPGKPQGPPQQEGNKPQGPPPPGKPQ. The observed mass of the whole peptide was 2725.3907, which is in good agreement with the theoretical mass of 2725.3900 (p=6.88 E-73). A peptide of the same sequence was synthesized and tested on the original LC-MS system used for discovery studies. The retention time, m/z and fragmentation pattern of the synthesized peptide on the LC-MS system used for discovery were virtually identical to those observed for the ion of interest originally identified in saliva.

The amino acid sequence of the candidate CFS biomarker is found within the amino acid sequence of the human salivary protein, Basic Proline Rich Protein 4 (PRB4_HUMAN, P10163). This sequence is not found within any other known animal, bacterial or plant proteins. In humans, PBR4 is known to be expressed only in the parotid and other saliva glands.

Analysis was performed to determine if levels of the biomarker candidate were dependent on various demographic factors. Table 3 shows the distribution of subjects according to use of medication, gender, race, smoking, regularity of menstruation, menopause status, and obesity. Table 3 indicates that the CFS subjects are more likely to be taking medication, be smokers and have irregular menstruation than the normal subjects group. Table 3 shows that age and BMI are not statistically different between CFS and normal subjects.

Because the distribution of demographic factors is quite different between CFS and normal subjects, it may be argued that the CFS biomarker candidate identified in this study is instead a measure of factors related specifically to sampling differences. In other words, the biomarker candidate may be associated with medication usage, smoking or other demographic factors. To examine this possibility, CFS subjects were compared statistically using t-test. These results are shown in Table 4. Medication status, gender, race, smoking status, quality of menstruation, menopause status, and obesity were not associated with CFS biomarker level. Similarly, relationship of age and BMI to biomarker level was also examined. In this case no association was observed between these factors and level of the biomarker in CFS subjects. Taken together these observations suggest that the CFS biomarker is associated only with CFS and not with other factors examined here.

The diagnostic utility was evaluated through construction of a Receiver Operating Characteristic (ROC) curve. The ROC curve is shown in FIG. 6. The calculated Area-Underthe-Curve (AUC) of this ROC curve is 0.935 with a standard error of 0.0319 and with a 95% confidence interval using the binomial exact method of 0.864 to 0.976. A diagnostic test that has ROC AUC of 1.0 indicates a test that is able to perfectly discriminate between subjects with the disease and those that do not have the disease. A ROC AUC of 0.5 indicates a test that does no better than random assignment of individuals to groups.

The present study confirms that saliva can be used for detection and evaluation of disease-associated biomarkers and that CFS modulates the expression of a very specific set of molecules. A battery of biochemical and bioinformatic tools was employed to identify a 2.7 kDa peptide present in the salivary samples from CFS patients, at levels much higher than that of normal subjects. By identifying the amino acid sequence of this peptide and performing a search against the non-redundant GenBank® database, it was determined that the 2.7 kDa peptide is a fragment of human salivary prolinerich protein 4 (PRB4).

PRB4 belongs to the family of human salivary proline-rich proteins (PRPs), which include six closely linked genes on

chromosome 12p13.2. All of the PRP genes are similar in structure, with complex electrophoretic patterns. Each PRP gene is approximately 4.0 kb in length and contains four exons, the third of which is entirely composed of 63-bp tandem repeats and encodes the proline-rich portion of the protein products. Exon 3 contains different numbers of tandem repeats in the different PRP genes. Variation in the numbers of these repeats is also responsible for length variations in different alleles of the PRB genes.

Currently, there are no specific biological or morphological biomarkers to establish per se the diagnosis of CFS. Its diagnosis is indefinite, and established through the exclusion of other diseases causing fatigue. Several studies have been conducted toward discovery of CFS biomarker(s), but the 15 14. Nater, U. M., et al., Attenuated morning salivary cortisol outcomes have been uncertain.

In conclusion, in the present study, a specific salivary biomarker was detected in subjects with CFS. Further studies are being designed to evaluate if the identified salivary 2.7 kDa peptide is not only a diagnostic biomarker but also a 20 prognostic tool as well. In addition, other roles for this peptide, e.g., as a potential mediator of disease development and its progression, are currently being assessed.

The above examples clearly illustrate the advantages of the invention. Although the present invention has been described 25 with reference to specific details of certain embodiments thereof, it is not intended that such details should be regarded as limitations upon the scope of the invention except as and to the extent that they are included in the accompanying claims.

Throughout this application, various patents, patent publications and non-patent publications are referenced. The disclosures of these patents, patent publications and non-patent publications in their entireties are incorporated by reference herein into this application in order to more fully describe the 35 state of the art to which this invention pertains.

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Basic Proline-rich Protein 2 (PRB2; UniProt: P02812)

MLLILLSVALLALSSAONLNEDVSQEESPSLIAGNPQGAPPQGGNKPQGP PSPPGKPQGPPPQGGNQPQGPPPPPGKPQGPPPQGGNKPQGPPPPGKPQG PPPOGDKSRSPRSPPGKPOGPPPOGGNOPOGPPPPPGKPOGPPPOGGNKP QGPPPPGKPQGPPPQGDNKSRSSRSPPGKPQGPPPQGGNQPQGPPPPPGK PQGPPPQGGNKPQGPPPPGKPQGPPPQGDNKSQSARSPPGKPQGPPPQGG ${\tt NQPQGPPPPGKPQGPPPQGGNKSQGPPPPGKPQGPPPQGGSKSRSSRSP}$ PGKPQGPPPQGGNQPQGPPPPPGKPQGPPPQGGNKPQGPPPPGKPQGPPP QGGSKSRSARSPPGKPQGPPQQEGNNPQGPPPPAGGNPQQPQAPPAGQPQ GPPRPPQGGRPSRPPQ

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5-mer peptides of P02812
                            PPPGK
                                               PPPPP
MLLIL
         SPPGK
                   POGDK
                                     PPOGG
                                               PPPPG
LLILL
         PPGKP
                   QGDKS
                            PPGKP
                                     PQGGN
LILLS
         PGKPO
                   GDKSR
                            PGKPO
                                     OGGNK
                                               PPPGK
ILLSV
         GKPOG
                   DKSRS
                            GKPQG
                                     GGNKP
                                               PPGKP
LLSVA
         KPOGP
                            KPOGP
                                     GNKPO
                                               PGKPO
LSVAL
         POGPE
                   SRSPR
                            POGPE
                                     NKPQG
                                               GKPQG
SVALL
         OGPPP
                   RSPRS
                            OGPPP
                                     KPOGP
                                               KPQGP
VALLA
         GPPPO
                   SPRSP
                            GPPPO
                                     PQGPP
                                               PQGPP
ALLAL
         PPPQG
                   PRSPP
                            PPPQG
                                     QGPPF
                                               OGPPP
LLALS
         PPQGG
                   RSPPG
                                     GPPPP
                                               GPPPQ
                            PPQGD
LALSS
                            PQGDN
         PQGGN
                   SPPGK
                                     PPPPG
                                               PPPQG
ALSSA
         QGGNQ
                            QGDNK
                   PPGKP
                                     PPPGK
                                               PPOGG
         GGNQP
LSSAQ
                   PGKPQ
                            GDNKS
                                     PPGKP
                                               PQGGN
SSAQN
                   GKPQG
                            DNKSR
                                               QGGNK
         GNOPO
                                     PGKPO
                            NKSRS
                                               GGNKS
SAONL
         NOPOG
                   KPOGP
                                     GKPOG
                            KSRSS
                                     KPOGP
                                               GNKSO
AONLN
         OPOGP
                   POGPP
         POGPP
                   OGPPP
                            SRSSR
                                     POGPP
                                               NKSOG
ONLNE
NLNED
         OGPPP
                   GPPPO
                            RSSRS
                                     OGPPP
                                               KSOGP
         GPPPP
                                     GPPPO
LNEDV
                   PPPOG
                            SSRSP
                                               SOGPP
                                               OGPPP
NEDVS
         PPPPP
                   PPOGG
                            SRSPP
                                     PPPOG
EDVSO
         PPPPG
                   POGGN
                            RSPPG
                                     PPOGD
                                               GPPPP
DVSOE
         PPPGK
                   OGGNO
                            SPPGK
                                     POGDN
                                               PPPPG
         PPGKP
                            PPGKP
                                               PPPGK
VSOFE
                   GGNOP
                                     OGDNK
                                               PPGKP
SOEES
         PGKPO
                   GNOPO
                            PGKPO
                                     GDNKS
                                               PGKPO
OFFSP
         GKPOG
                   NOPOG
                            GKPOG
                                     DNKSO
EESPS
         KPOGP
                   OPOGP
                            KPQGP
                                     NKSOS
                                               GKPQG
ESPSL
         POGPP
                   POGPP
                            POGPP
                                     KSQSA
                                               KPOGP
SPSLIT
         OGPPP
                   OGPPP
                            OGPPP
                                     SOSAR
                                               POGPP
PSLIA
         GPPPO
                   GPPPP
                            GPPPO
                                     OSARS
                                               OGPPP
SLIAG
         PPPOG
                   PPPPP
                            PPPOG
                                     SARSP
                                               GPPPO
LIAGN
         PPOGG
                   PPPPG
                            PPOGG
                                     ARSPP
                                               PPPOG
IAGNP
                   PPPGK
         PQGGN
                            POGGN
                                     RSPPG
                                               PPQGG
AGNPQ
         QGGNK
                   PPGKP
                            OGGNO
                                     SPPGK
                                               PQGGS
GNPOG
         GGNKP
                   PGKPO
                            GGNQP
                                     PPGKP
                                               OGGSK
NPQGA
         GNKPQ
                   GKPQG
                            GNQPQ
                                     PGKPQ
                                               GGSKS
PQGAP
         NKPOG
                   KPOGP
                            NQPQG
                                     GKPQG
                                               GSKSR
QGAPP
         KPQGP
                   PQGPP
                            QPQGP
                                      KPQGP
                                               SKSRS
GAPPO
         PQGPP
                   OGPPP
                            PQGPP
                                     PQGPP
                                               KSRSS
APPQG
         QGPPP
                   GPPPQ
                            QGPPP
                                      QGPPP
                                               SRSSR
PPOGG
         GPPPP
                   PPPOG
                            GPPPP
                                      GPPPQ
                                               RSSRS
PQGGN
         PPPPG
                   PPQGG
                            PPPPP
                                     PPPQG
                                               SSRSP
QGGNK
         PPPGK
                   PQGGN
                            PPPPG
                                     PPQGG
                                               SRSPP
                                               RSPPG
GGNKP
         PPGKP
                   OGGNK
                            PPPGK
                                     POGGN
GNKPQ
         PGKPQ
                   GGNKP
                            PPGKP
                                     QGGNQ
                                               SPPGK
NKPOG
         GKPOG
                   GNKPO
                            PGKPO
                                      GGNOP
                                               PPGKP
KPQGP
         KPQGP
                   NKPQG
                            GKPOG
                                     GNQPQ
                                               PGKPQ
POGPP
         POGPE
                   KPQGP
                            KPQGP
                                     NQPQG
                                               GKPOG
OGPPS
         OGPPP
                   PQGPP
                            PQGPP
                                     QPQGP
                                               KPQGP
GPPSP
         GPPPO
                   OGPPP
                            OGPPP
                                      PQGPP
                                               PQGPP
                                     QGPPP
PPSPP
         PPPQG
                   GPPPP
                            GPPPO
                                               OGPPP
```

PPPPG

GRPSR

PPPOG

GPPPP

GPPPO

PSPPG

PPPQG

PPOGD

SARSP

62 -continued

RPSRP

PSRPP

SRPPO

PQGGN RSPPG QGGNQ SPPGK GGNQP PPGKP GNOPO PGKPQ NOPOG GKPOG QPQGP KPQGP PQGPP POGPP QGPPP OGPPO GPPPP GPPQQ PPPPP PPQQE PPPPG POOEG PPPGK

ARSPP

QQEGN PPGKP QEGNN PGKPO EGNNP GKPQG GNNPQ KPQGP NNPQG POGPP NPOGP OGPPP POGPP

GPPPO OGPPP PPPOG GPPPP PPOGG PPPPA POGGN PPPAG OGGNK PPAGG GGNKP PAGGN GNKPO AGGNP NKPOG GGNPO

GNPOO

NPOOP

AGOPO

GQPQG

20

25 OGPPP POOPO GPPPP QQPQA PPPPG OPOAP PPPGK PQAPP PPGKP OAPPA PGKPQ APPAG GKPQG PPAGO KPOGP PAGQP

KPOGP

POGPP

PQGPP

QGPPP

GPPPQ QPQGP PPPQG POGPP PPQGG QGPPR POGGS GPPRP QGGSK PPRPP GGSKS PRPPC GSKSR RPPQG SKSRS PPQGG

KSRSA POGGR SRSAR OGGRP RSARS

Basic Proline-rich Protein 3 (PRB3: UniProt:

MLLILLSVALLALSSAQSLNEDVSQEESPSVISGKPEGRRPQGGNQPQRT ${\tt PPPPGKPEGRPPQGGNQSQGPPPRPGKPEGPPPQGGNQSQGPPPRPGKPE}$ ${\tt GQPPQGGNQSQGPPPRPGKPEGPPPQGGNQSQGPPPRPGKPEGPPPQGGN}$ QSQGPPPHPGKPEGPPPQGGNQSQGPPPRPGKPEGPPPQGGNQSQGPPPR PGKPEGPPPQGGNQSQGPPPRPGKPEGSPSQGGNKPQGPPPHPGKPQGPP PQEGNKPQRPPPPGRPQGPPPPGGNPQQPLPPPAGKPQGPPPPPQGGRPH RPPOGOPPO

50 5-mer peptides of Q04118 MLLIL PPPGK GPPPH EGPPP KPORP PPOGG PPPHP PORPP LLILL PPGKP POGGN **GPPPO** LILLS PGKPE OGGNO PPHPG PPPOG ORPPP RPPPP ILLSV GGNOS PHPGK GKPEG PPOGG LLSVA KPEGR GNOSO **HPGKP** POGGN PPPPG LSVAL PEGRP NOSOG PGKPE OGGNO PPPGR SVALL EGRPP **OSOGP** GKPEG GGNOS PPGRP VALLA GRPPO SOGPP KPEGP GNOSO PGRPO ALLAL RPPOG OGPPP PEGPP NOSOG GRPOG LLALS PPQGG GPPPR EGPPP OSOGP RPOGP LALSS POGGN PPPRP **GPPPO** SOGPP POGPP 60 ALSSA OGGNO PPRPG PPPOG OGPPP OGPPP LISSAO GGNOS PRPGK PPOGG GPPPR GPPPP SSAOS GNOSO RPGKP PQGGN PPPRP PPPPG PPPGG SAQSL NQSQG PGKPE QGGNQ PPRPG AQSLN QSQGP GKPEG GGNQS PRPGK PPGGN PGGNP QSLNE SOGPP KPEGP GNOSO RPGKP SLNED PEGPP QGPPP NOSQG PGKPE GGNPQ LNEDV GPPPR EGPPP GKPEG GNPQQ QSQGP

			ntinued						-co	ntinued	i.	
NEDVS	PPPRP	GPPPQ	SQGPP	KPEGS	NPQQP		RPQGG	GPPPH	ERPPP	KPQGP		
EDVSQ	PPRPG	PPPQG	QGPPP	PEGSP	PQQPL		PQGGN	PPPHP	RPPPQ	PQGPP		
DVSQE	PRPGK	PPQGG	GPPPR	EGSPS	QQPLP		QGGNQ	PPHPG	PPPQG	QGPPP		
VSQEE	RPGKP	PQGGN	PPPRP	GSPSQ	QPLPP				-			
SQEES	PGKPE	QGGNQ	PPRPG	SPSQG	PLPPP	5	GGNQP	PHPGK	PPQGG	GPPPP		
QEESP	GKPEG	GGNQS	PRPGK	PSQGG	LPPPA		GNQPQ	HPGKP	PQGGN	PPPPG		
							NQPQR	PGKPE	QGGNQ	PPPGK		
EESPS	KPEGP	GNQSQ	RPGKP	SQGGN	PPPAG		QPQRP	GKPER	GGNQS	PPGKP		
ESPSV	PEGPP	NQSQG	PGKPE	QGGNK	PPAGK		PQRPP	KPERP	GNQSQ	PGKPQ		
SPSVI	EGPPP	QSQGP	GKPEG	GGNKP	PAGKP		QRPPP	PERPP	NQSQG	GKPQG		
PSVIS	GPPPQ	SQGPP	KPEGP	GNKPQ	AGKPQ					-		
SVISG	PPPQG	QGPPP	PEGPP	NKPQG	GKPQG	10	RPPPP	ERPPP	QSQGP	KPQGP		
VISGK	PPQGG	GPPPR	EGPPP	KPQGP	KPQGP		PPPPP	RPPPQ	SQGPP	PQGPP		
ISGKP	PQGGN	PPPRP	GPPPQ	PQGPP	PQGPP		PPPPG	PPPQG	QGPPP	QGPPP		
SGKPE	QGGNQ	PPRPG	PPPQG	QGPPP	QGPPP							
							Pagig	Proline-r	ich Prote	ain 4 (DE	DA. IIniD	rot.
GKPEG	GGNQS	PRPGK	PPQGG	GPPPH	GPPPP				ICH PIOCE	atii 4 (br	CB4; OHIP	LOC:
KPEGR	GNQSQ	RPGKP	PQGGN	PPPHP	PPPPP		P10163	•				
PEGRR	NQSQG	PGKPE	QGGNQ	PPHPG	PPPPQ	15	MLLILL	SVALLALSS.	AESSSEDVS	SQEESLFLI	SGKPEGRR	PQGGNQPQRP
EGRRP	QSQGP	GKPEG	GGNQS	PHPGK	PPPQG	10	PPPPGK	PQGPPPQGG:	NQSQGPPPI	PPGKPEGRE	PQGGNQSQ	GPPPHPGKPE
GRRPQ	SQGPP	KPEGP	GNQSQ	HPGKP	PPQGG		RPPPOG	GNOSOGPPP	HPGKPESRI	PPOGGHOSO	GPPPTPGK	PEGPPPQGGN
RRPQG	QGPPP	PEGPP	NQSQG	PGKPQ	PQGGR							GNQSHRPPPP
RPQGG	GPPPR	EGPPP	QSQGP	GKPQG	QGGRP							
PQGGN	PPPRP	GPPPQ	SQGPP	KPQGP	GGRPH							SPPGKPQGPP
							QQEGNK	PQGPPPPGK	PQGPPPPG	INPQQPQAF	PAGKPQGP	PPPPQGGRPP
QGGNQ	PPRPG	PPPQG	QGPPP	PQGPP	GRPHR	20	RPAQGQ	QPPQ				
GGNQP	PRPGK	PPQGG	GPPPR	QGPPP	RPHRP							
GNQPQ	RPGKP	PQGGN	PPPRP	GPPPQ	PHRPP		5-mer	peptides	of P10163	2		
NQPQR	PGKPE	QGGNQ	PPRPG	PPPQE	HRPPQ						EDDDD	KDOGD
QPQRT	GKPEG	GGNQS	PRPGK	PPQEG	RPPQG		MLLIL	PPPGK	PPQGG	GTPPP	ERPPP	KPQGP
PORTP	KPEGQ	GNQSQ	RPGKP	PQEGN	PPQGQ		LLILL	PPGKP	PQGGN	TPPPP	RPPPQ	PQGPP
QRTPP	PEGQP	NQSQG	PGKPE	QEGNK	PQGQP		LILLS	PGKPQ	QGGNQ	PPPPG	PPPQG	QGPPP
	EGOPP		GKPEG	-		25	ILLSV	GKPQG	GGNQS	PPPGK	PPQGG	GPPPP
RTPPP	~	QSQGP		EGNKP	QGQPP	23	LLSVA	KPQGP	GNQSQ	PPGKP	PQGGN	PPPPG
TPPPP	GQPPQ	SQGPP	KPEGP	GNKPQ	GQPPQ							
PPPPG	QPPQG	QGPPP	PEGPP	NKPQR			LSVAL	PQGPP	NQSQG	PGKPE	QGGNQ	PPPGK
							SVALL	QGPPP	QSQGP	GKPEG	GGNQS	PPGKP
Basic P	roline-r	ich Prote	in 4 (PR	B4: UniP	rot:		VALLA	GPPPO	SQGPP	KPEGR	GNQSQ	PGKPQ
E9PAL0)			•	,			ALLAL	PPPQG	QGPPP	PEGRP	NQSQG	GKPQG
	373T.T.3T.CC	V ECCCEUM	OPPCI.DI.T	CCKDECED	PQGGNQPQRP	20						
						30	LLALS	PPQGG	GPPPH	EGRPP	QSQGP	KPQGP
					GPPPHPGKPE		LALSS	PQGGN	PPPHP	GRPPQ	SQGPP	PQGPP
RPPPQGG	NQSQGTPP:	PPGKPERPF	PQGGNQSH	RPPPPPGK	PERPPPQGGN		ALSSA	QGGNQ	PPHPG	RPPQG	QGPPP	QGPPP
QSQGPPP	HPGKPEGP	PPQEGNKSF	RSARSPPGK	PQGPPQQE	GNKPQGPPPP		LSSAE	GGNQS	PHPGK	PPQGG	GPPPH	GPPPP
GKPQGPP	PAGGNPQQ	PQAPPAGKE	QGPPPPPQ	GGRPPRPA	QGQQPPQ		SSAES					
~	~~	~						GNQSQ	HPGKP	PQGGN	PPPHP	PPPPG
5-mer n	entides	of E9PALO)				SAESS	NQSQG	PGKPE	QGGNQ	PPHPG	PPPGG
MLLIL	PPPGK	PPQGG	GPPPH	GPPPA		35	AESSS	QSQGP	GKPES	GGNQS	PHPGK	PPGGN
							ESSSE	SQGPP	KPESR	GNQSQ	HPGKP	PGGNP
LLILL	PPGKP	PQGGN	PPPHP	PPPAG			SSSED	QGPPP	PESRP	NQSQG	PGKPE	GGNPQ
LILLS	PGKPQ	QGGNQ	PPHPG	PPAGG								
ILLSV	GKPQG	GGNQS	PHPGK	PAGGN			SSEDV	GPPPP	ESRPP	QSQGP	GKPEG	GNPQQ
LLSVA	KPQGP	GNQSQ	HPGKP	AGGNP			SEDVS	PPPPP	SRPPQ	SQGPP	KPEGP	NPQQP
LSVAL	PQGPP	NOSQG	PGKPE	GGNPQ			EDVSQ	PPPPG	RPPQG	QGPPP	PEGPP	PQQPQ
SVALL	QGPPP	QSQGT	GKPEG	GNPQQ		40	DVSQE	PPPGK	PPQGG	GPPPH	EGPPP	QQPQA
							VSQEE	PPGKP	POGGH	PPPHP	GPPPQ	QPQAP
VALLA	GPPPQ	SQGTP	KPEGP	NPQQP					-			
ALLAL	PPPQG	QGTPP	PEGPP	PQQPQ			SQEES	PGKPE	QGGHQ	PPHPG	PPPQE	PQAPP
LLALS	PPQGG	GTPPP	EGPPP	QQPQA			QEESL	GKPEG	GGHQS	PHPGK	PPQEG	QAPPA
LALSS	PQGGN	TPPPP	GPPPQ	QPQAP			EESLF	KPEGR	GHQSQ	HPGKP	PQEGN	APPAG
ALSSA	QGGNQ	PPPPG	PPPQE	PQAPP			ESLFL	PEGRP	HQSQG	PGKPE	QEGNK	PPAGK
LSSAE	GGNQS	PPPGK	PPQEG	QAPPA		45						
SSAES	GNQSQ	PPGKP	PQEGN	APPAG			SLFLI	EGRPP	QSQGP	GKPER	EGNKS	PAGKP
SAESS	NOSOG	PGKPE	QEGNK	PPAGK			LFLIS	GRPPQ	SQGPP	KPERP	GNKSR	AGKPQ
							FLISG	RPPQG	QGPPP	PERPP	NKSRS	GKPQG
AESSS	QSQGP	GKPER	EGNKS	PAGKP			LISGK	PPQGG	GPPPT	ERPPP	KSRSA	KPQGP
ESSSE	SQGPP	KPERP	GNKSR	AGKPQ			ISGKP	PQGGN	PPPTP	RPPPQ	SRSAR	PQGPP
SSSED	QGPPP	PERPP	NKSRS	GKPQG			SGKPE		PPTPG	PPPQG		QGPPP
SSEDV	GPPPP	ERPPP	KSRSA	KPQGP		50		QGGNQ		-	RSARS	
SEDVS	PPPPP	RPPPQ	SRSAR	PQGPP			GKPEG	GGNQS	PTPGK	PPQGG	SARSP	GPPPP
EDVSQ	PPPPG	PPPQG	RSARS	QGPPP			KPEGR	GNQSQ	TPGKP	PQGGN	ARSPP	PPPPP
DVSQE	PPPGK	PPQGG	SARSP	GPPPP			PEGRR	NQSQG	PGKPE	QGGNQ	RSPPG	PPPPQ
							EGRRP	QSQGP	GKPEG	GGNQS	SPPGK	PPPQG
VSQEE	PPGKP	PQGGN	ARSPP	PPPPP								
SQEES	PGKPE	QGGNQ	RSPPG	PPPPQ			GRRPQ	SQGPP	KPEGP	GNQSH	PPGKP	PPQGG
QEESL	GKPEG	GGNQS	SPPGK	PPPQG		55	RRPQG	QGPPP	PEGPP	NQSHR	PGKPQ	PQGGR
EESLF	KPEGR	GNQSH	PPGKP	PPQGG		55	RPQGG	GPPPH	EGPPP	QSHRP	GKPQG	QGGRP
ESLFL	PEGRP	NOSHR	PGKPQ	PQGGR			POGGN	PPPHP	GPPPQ	SHRPP	KPQGP	GGRPP
SLFLI	EGRPP	QSHRP	GKPQG	QGGRP			QGGNQ	PPHPG	PPPQG	HRPPP	PQGPP	GRPPR
LFLIS	GRPPQ	SHRPP	KPQGP	GGRPP								
							GGNQP	PHPGK	PPQGG	RPPPP	QGPPQ	RPPRP
FLISG	RPPQG	HRPPP	PQGPP	GRPPR			GNQPQ	HPGKP	PQGGN	PPPPP	GPPQQ	PPRPA
LISGK	PPQGG	RPPPP	QGPPQ	RPPRP		60	NQPQR	PGKPE	QGGNQ	PPPPG	PPQQE	PRPAQ
ISGKP	PQGGN	PPPPP	GPPQQ	PPRPA		00	QPQRP	GKPER	GGNQS	PPPGK	PQQEG	RPAQG
SGKPE	QGGNQ	PPPPG	PPQQE	PRPAQ								
GKPEG	GGNQS	PPPGK	PQQEG	RPAQG			PQRPP	KPERP	GNQSQ	PPGKP	QQEGN	PAQGQ
KPEGR	GNQSQ	PPGKP	QQEGN	PAQGQ			QRPPP	PERPP	NQSQG	PGKPE	QEGNK	AQGQQ
							RPPPP	ERPPP	QSQGT	GKPER	EGNKP	QGQQP
PEGRR	NOSOG	PGKPE	QEGNK	AQGQQ			PPPPP					
EGRRP	QSQGP	GKPER	EGNKP	QGQQP				RPPPQ	SQGTP	KPERP	GNKPQ	GQQPP
GRRPQ	SQGPP	KPERP	GNKPQ	GQQPP		65	PPPPG	PPPQG	QGTPP	PERPP	NKPQG	QQPPQ
RRPQG	QGPPP	PERPP	NKPQG	QQPPQ								

GRRPQ RRPQG

QGPPP

PERPP

NKPQG

QQPPQ

15

45

50

55

60

65

-continued Salivary acidic proline-rich phosphoprotein 1/2(PRH1/PRH2; UniProt: P02810)

MLLILLSVALLAFSSAQDLDEDVSQEDVPLVISDGGDSEQFIDEERQGPP LGGQQSQPSAGDGNQDDGPQQGPPQQGGQQQQGPPPPQGKPQGPPQQGGH ${\tt PPPPQGRPQGPPQQGGHPRPPRGRPQGPPQQGGHQQGPPPPPPGKPQGPP}$ PQGGRPQGPPQGQSPQ

5-mer	peptides	of P02810		
MLLIL	GGQQS	PPQGR	GRPQG	
LLILL	GQQSQ	PQGRP	RPQGP	
LILLS	QQSQP	QGRPQ	PQGPP	
ILLSV	QSQPS	GRPQG	QGPPQ	
LLSVA	SQPSA	RPQGP	GPPQG	
LSVAL	QPSAG	PQGPP	PPQGQ	
SVALL	PSAGD	QGPPQ	PQGQS	
VALLA	SAGDG	GPPQQ	QGQSP	
ALLAF	AGDGN	PPQQG	GQSPQ	
LLAFS	GDGNQ	PQQGG		
LAFSS	DGNQD	QQGGH		
AFSSA	GNQDD	QGGHP		
FSSAQ	NQDDG	GGHPR		
SSAQD	QDDGP	GHPRP		
SAQDL	DDGPQ	HPRPP		
AQDLD	DGPQQ	PRPPR		
QDLDE	GPQQG	RPPRG		
DLDED	PQQGP	PPRGR		
LDEDV	QQGPP	PRGRP		
DEDVS	QGPPQ	RGRPQ		
EDVSQ	GPPQQ	GRPQG		
DVSQE	PPQQG	RPQGP		
VSQED	PQQGG	PQGPP		
SQEDV	QQGGQ	QGPPQ		
QEDVP	QGGQQ	GPPQQ		
EDVPL	GGQQQ	PPQQG		
DVPLV	GQQSQ	PQQGG		
VPLVI	QQQQG	QQGGH		
PLVIS	QQQGP	QGGHQ		
LVISD	QQGPP	GGHQQ		
VISDG	QGPPP	GHQQG		
ISDGG	GPPPP	HQQGP		
SDGGD	PPPPQ	QQGPP		
DGGDS	PPPQG	QGPPP		
GGDSE	PPQGK	GPPPP		
GDSEO	PQGKP	PPPPP		
DSEQF	QGKPQ	PPPPP		
SEQFI	GKPQG	PPPPG		
EQFID		PPPGK		
	KPQGP			
QFIDE	PQGPP	PPGKP		
FIDEE	QGPPQ	PGKPQ		
IDEER	GPPQQ	GKPQG		
DEERQ	PPQQG	KPQGP		
EERQG	PQQGG	PQGPP		
ERQGP	QQGGH	QGPPP		
RQGPP	QGGHP	GPPPQ		
QGPPL	GGHPP	PPPQG		
GPPLG	GHPPP	PPQGG		
PPLGG	HPPPP	PQGGR		
PLGGQ	PPPPQ	QGGRP		
- Z	2	~		

TABLE 2

GGRPQ

LGGQQ

PPPQG

Basic Proline-rich Protein 1 (PRB1; UniProt: P04280)
MLLILLSVALLALSSAQNLNEDVSQEESPSLIAGNPQGPSPQGGNKPQGP
PPPPGKPQGPPPQGGNKPQGPPPPGKPQGPPPQGDKSRSPRSPPGKPQGP
PPQGGNQPQGPPPPGKPQGPPPQGGNKPQGPPPPGKPQGPPPQGDKSQS
PRSPPGKPQGPPPQGGNQPQGPPPPPGKPQGPPPQGGNKPQGPPPPGKPQ
GPPPQGDKSQSPRSPPGKPQGPPPQGGNQPQGPPPPPGKPQGPPQQGGNR

TABLE 2-continued

Basic Proline-rich Protein 1 (PRB1; UniProt: P04280)

 $\verb"PQGPPPGKPQGPPPQGDKSRSPQSPPGKPQGPPPQGGNQPQGPPPPPGK"$ $\verb"PQGPPPQGGNKPQGPPPGKPQGPPAQGGSKSQSARAPPGKPQGPPQQEG"$

 ${\tt NNPQGPPPPAGGNPQQPQAPPAGQPQGPPRPPQGGRPSRPPQ}$

TABLE 3

Distribution of CFS and normal subjects with regard to demographic and other factors. Expressed as percentage demographic and other factors. Expressed as percentage where CFS n = 46 and normal subject n = 45. Chi-square statistic. Body weight is defined as normal, BMI between 18.5 to 24.9, overweight 25.0 to 29.9, and obese greater than 30 (www.cdc.gov/obesity/adult/defining.html). A smoker is defined as anyone who answered yes to the question "Have you ever smoked cigarettes regularly?"

	"Have you	ever smoked eig	arettes regularly	<i>?''</i>
		CFS	Normal	p-value
	Medication			0.001
1	Not Medicated	43.5	97.8	
1	Medicated	56.5	2.2	
; (Gender			0.059
]	Female	78.3	69.2	
ľ	Male	21.7	30.8	
]	Race			0.385
1	American Indian	4.3	0.0	
]	Black	15.2	15.6	
) (Other	2.2	0.0	
	White	78.3	84.4	
5	Smoking			0.005
5	Smoker	67.4	37.8	
1	Non-Smoker	32.6	62.2	
1	Menstruation			0.016
. 1	Normal	16.7	44.4	
j	rregular	83.3	55.6	
	Menopause			0.109
•	Yes	61.1	40.7	
1	No	38.9	59.3	
(Obesity			0.127
	Normal	17.4	33.3	
) (Overweight	39.1	40.0	
	Obese	43.5	26.7	
2	Age	50.4	48.0	0.218
	BMI	28.6	27.1	0.100

TABLE 4

CFS Subjects compared	Mean	$^{\mathrm{SD}}$	p-valu
Medication	_		
Not Medicated (n = 20) Medicated (n = 26) Gender	52160 47736	19727 20737	0.465
Female (n = 36) Male (n = 10) Race	50684 45969	21557 14632	0.43
White (n = 36) Other (n = 10) Smoking	49889 48833	21060 17743	0.875
Smoker (n = 31) Non-Smoker (n = 15) Menstruation (female only)	47053 55047	18888 22387	0.25
Irregular (n = 30) Normal (n = 6)	55830 49655	20599 21935	0.525

TABLE 4-continued

68TABLE 4-continued

Comparison of CFS subjects	CFS biomarke	r level withi	n group		Comparison of CFS subjects C	r level within	evel within group	
CFS Subjects compared	Mean SD		p-value	5 -	CFS Subjects compared	Mean	SD	p-value
Menopause (female only)				Obesity				
Yes (n = 22) No (n = 14)	48307 54420	23014 19262	0.397	_	Normal (n = 8) Overweight or Obese (n = 38)	52905 48976	20554 20339	0.633

SEQUENCE LISTING

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Gly Pro Pro Ser Pro Pro Gly Lys Pro Gln Gly Pro Pro Pro Gln Gly
       55
Gly Asn Gln Pro Gln Gly Pro Pro Pro Pro Gly Lys Pro Gln Gly
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											-	con	tin	ued	
65					70					75					80
Pro	Pro	Pro	Gln	Gly 85	Gly	Asn	Lys	Pro	Gln 90	Gly	Pro	Pro	Pro	Pro 95	Gly
Lys	Pro	Gln	Gly 100	Pro	Pro	Pro	Gln	Gly 105	Asp	Lys	Ser	Arg	Ser 110	Pro	Arg
Ser	Pro	Pro 115	Gly	Lys	Pro	Gln	Gly 120	Pro	Pro	Pro	Gln	Gly 125	Gly	Asn	Gln
Pro	Gln 130	Gly	Pro	Pro	Pro	Pro 135	Pro	Gly	Lys	Pro	Gln 140	Gly	Pro	Pro	Pro
Gln 145	Gly	Gly	Asn	Lys	Pro 150	Gln	Gly	Pro	Pro	Pro 155	Pro	Gly	Lys	Pro	Gln 160
Gly	Pro	Pro	Pro	Gln 165	Gly	Asp	Asn	Lys	Ser 170	Arg	Ser	Ser	Arg	Ser 175	Pro
Pro	Gly	ГЛа	Pro 180	Gln	Gly	Pro	Pro	Pro 185	Gln	Gly	Gly	Asn	Gln 190	Pro	Gln
Gly	Pro	Pro 195	Pro	Pro	Pro	Gly	Lys 200	Pro	Gln	Gly	Pro	Pro 205	Pro	Gln	Gly
Gly	Asn 210	Lys	Pro	Gln	Gly	Pro 215	Pro	Pro	Pro	Gly	Lys 220	Pro	Gln	Gly	Pro
Pro 225	Pro	Gln	Gly	Asp	Asn 230	Lys	Ser	Gln	Ser	Ala 235	Arg	Ser	Pro	Pro	Gly 240
ГÀв	Pro	Gln	Gly	Pro 245	Pro	Pro	Gln	Gly	Gly 250	Asn	Gln	Pro	Gln	Gly 255	Pro
Pro	Pro	Pro	Pro 260	Gly	Lys	Pro	Gln	Gly 265	Pro	Pro	Pro	Gln	Gly 270	Gly	Asn
ГÀв	Ser	Gln 275	Gly	Pro	Pro	Pro	Pro 280	Gly	Lys	Pro	Gln	Gly 285	Pro	Pro	Pro
Gln	Gly 290	Gly	Ser	Lys	Ser	Arg 295	Ser	Ser	Arg	Ser	Pro 300	Pro	Gly	Lys	Pro
Gln 305	Gly	Pro	Pro	Pro	Gln 310	Gly	Gly	Asn	Gln	Pro 315	Gln	Gly	Pro	Pro	Pro 320
Pro	Pro	Gly	Lys	Pro 325	Gln	Gly	Pro	Pro	Pro 330	Gln	Gly	Gly	Asn	1335	Pro
Gln	Gly	Pro	Pro 340	Pro	Pro	Gly	Lys	Pro 345	Gln	Gly	Pro	Pro	Pro 350	Gln	Gly
Gly	Ser	355	Ser	Arg	Ser	Ala	Arg 360	Ser	Pro	Pro	Gly	Lys 365	Pro	Gln	Gly
Pro	Pro 370	Gln	Gln	Glu	Gly	Asn 375	Asn	Pro	Gln	Gly	Pro 380	Pro	Pro	Pro	Ala
Gly 385	Gly	Asn	Pro	Gln	Gln 390	Pro	Gln	Ala	Pro	Pro 395	Ala	Gly	Gln	Pro	Gln 400
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e 210) _{>}	יז סי	ои с	5											
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	2 > T 3 > OI		PRT ISM:	Homo	sa]	piens	3								
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Met	Leu	Leu	Ile	Leu											
1				5											
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Pro Arg Pro Pro Gln
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Gln Gly Gly Arg Pro
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Gly Gly Arg Pro Ser
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Gly Arg Pro Ser Arg
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Gln Ser Leu Asn Glu Asp Val Ser Gln Glu Glu Ser Pro Ser Val Ile
Ser Gly Lys Pro Glu Gly Arg Arg Pro Gln Gly Gly Asn Gln Pro Gln
Arg Thr Pro Pro Pro Pro Gly Lys Pro Glu Gly Arg Pro Pro Gln Gly
Gly Asn Gln Ser Gln Gly Pro Pro Pro Arg Pro Gly Lys Pro Glu Gly
Pro Pro Pro Gln Gly Gly Asn Gln Ser Gln Gly Pro Pro Pro Arg Pro
                            90
Gly Lys Pro Glu Gly Gln Pro Pro Gln Gly Gly Asn Gln Ser Gln Gly
          100
                             105
Pro Pro Pro Arg Pro Gly Lys Pro Glu Gly Pro Pro Pro Gln Gly Gly
Asn Gln Ser Gln Gly Pro Pro Pro Arg Pro Gly Lys Pro Glu Gly Pro
           135
Pro Pro Gln Gly Gly Asn Gln Ser Gln Gly Pro Pro Pro His Pro Gly
Lys Pro Glu Gly Pro Pro Pro Gln Gly Gly Asn Gln Ser Gln Gly Pro
Pro Pro Arg Pro Gly Lys Pro Glu Gly Pro Pro Pro Gln Gly Gly Asn
Gln Ser Gln Gly Pro Pro Pro Arg Pro Gly Lys Pro Glu Gly Pro Pro
Pro Gln Gly Gly Asn Gln Ser Gln Gly Pro Pro Pro Arg Pro Gly Lys
               215
Pro Glu Gly Ser Pro Ser Gln Gly Gly Asn Lys Pro Gln Gly Pro Pro
Pro His Pro Gly Lys Pro Gln Gly Pro Pro Pro Gln Glu Gly Asn Lys
                        250
Pro Gln Arg Pro Pro Pro Pro Gly Arg Pro Gln Gly Pro Pro Pro
Gly Gly Asn Pro Gln Gln Pro Leu Pro Pro Pro Ala Gly Lys Pro Gln
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Gly Pro Pro Pro Pro Gln Gly Gly Arg Pro His Arg Pro Pro Gln
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Ile Leu Leu Ser Val
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Leu Leu Ala Leu Ser
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Ala Leu Ser Ser Ala
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Leu Ser Ser Ala Gln
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Ala Gln Ser Leu Asn
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Leu Asn Glu Asp Val
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Gly Arg Arg Pro Gln
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Asn Gln Pro Gln Arg
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Pro Pro Pro Gly Lys
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Gln Ser Gln Gly Pro
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Ser Gln Gly Pro Pro
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Gln Gly Pro Pro Pro
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Gly Pro Pro Pro Arg
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Pro Pro Pro Arg Pro
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Lys Pro Gln Arg Pro
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Glu Ser Ser Ser Glu Asp Val Ser Gln Glu Glu Ser Leu Phe Leu Ile
Ser Gly Lys Pro Glu Gly Arg Arg Pro Gln Gly Gly Asn Gln Pro Gln
Arg Pro Pro Pro Pro Gly Lys Pro Gln Gly Pro Pro Pro Gln Gly
Gly Asn Gln Ser Gln Gly Pro Pro Pro Pro Pro Gly Lys Pro Glu Gly 65 70 75 80
Arg Pro Pro Gln Gly Gly Asn Gln Ser Gln Gly Pro Pro Pro His Pro
                         90
Gly Lys Pro Glu Arg Pro Pro Pro Gln Gly Gly Asn Gln Ser Gln Gly
Thr Pro Pro Pro Pro Gly Lys Pro Glu Arg Pro Pro Pro Gln Gly Gly
                          120
Asn Gln Ser His Arg Pro Pro Pro Pro Gly Lys Pro Glu Arg Pro
Pro Pro Gln Gly Gly Asn Gln Ser Gln Gly Pro Pro Pro His Pro Gly
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Lys Pro Glu Gly Pro Pro Pro Gln Glu Gly Asn Lys Ser Arg Ser Ala
               165
                                  170
Arg Ser Pro Pro Gly Lys Pro Gln Gly Pro Pro Gln Gln Glu Gly Asn
Lys Pro Gln Gly Pro Pro Pro Gly Lys Pro Gln Gly Pro Pro Pro
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Ala Gly Gly Asn Pro Gln Gln Pro Gln Ala Pro Pro Ala Gly Lys Pro
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Gln Gly Pro Pro Pro Pro Gln Gly Gly Arg Pro Pro Arg Pro Ala
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Lys Pro Glu Gly Arg
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Glu Ser Ser Ser Glu Asp Val Ser Gln Glu Glu Ser Leu Phe Leu Ile
Ser Gly Lys Pro Glu Gly Arg Arg Pro Gln Gly Gly Asn Gln Pro Gln
Arg Pro Pro Pro Pro Gly Lys Pro Gln Gly Pro Pro Pro Gln Gly
          55
Gly Asn Gln Ser Gln Gly Pro Pro Pro Pro Pro Gly Lys Pro Glu Gly
Arg Pro Pro Gln Gly Gly Asn Gln Ser Gln Gly Pro Pro Pro His Pro
Gly Lys Pro Glu Arg Pro Pro Pro Gln Gly Gly Asn Gln Ser Gln Gly
Pro Pro Pro His Pro Gly Lys Pro Glu Ser Arg Pro Pro Gln Gly Gly
                         120
His Gln Ser Gln Gly Pro Pro Pro Thr Pro Gly Lys Pro Glu Gly Pro
                      135
Pro Pro Gln Gly Gly Asn Gln Ser Gln Gly Thr Pro Pro Pro Gly
Lys Pro Glu Gly Arg Pro Pro Gln Gly Gly Asn Gln Ser Gln Gly Pro
                                   170
Pro Pro His Pro Gly Lys Pro Glu Arg Pro Pro Pro Gln Gly Gly Asn
Gln Ser His Arg Pro Pro Pro Pro Pro Gly Lys Pro Glu Arg Pro Pro
Pro Gln Gly Gly Asn Gln Ser Gln Gly Pro Pro Pro His Pro Gly Lys
Pro Glu Gly Pro Pro Pro Gln Glu Gly Asn Lys Ser Arg Ser Ala Arg
Ser Pro Pro Gly Lys Pro Gln Gly Pro Pro Gln Gln Glu Gly Asn Lys
Pro Gln Gly Pro Pro Pro Pro Gly Lys Pro Gln Gly Pro Pro Pro Pro Pro 260 265 270
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Gly Gln Gln Pro Pro Gln
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Ser Asp Gly Gly Asp Ser Glu Gln Phe Ile Asp Glu Glu Arg Gln Gly
Pro Pro Leu Gly Gly Gln Gln Ser Gln Pro Ser Ala Gly Asp Gly Asn
Gln Asp Asp Gly Pro Gln Gln Gly Pro Pro Gln Gln Gly Gly Gln Gln
Gln Gln Gly Pro Pro Pro Gln Gly Lys Pro Gln Gly Pro Pro Gln
Gln Gly Gly His Pro Pro Pro Gln Gly Arg Pro Gln Gly Pro Pro
                   105
Gln Gln Gly Gly His Pro Arg Pro Pro Arg Gly Arg Pro Gln Gly Pro
                           120
Pro Gln Gln Gly Gly His Gln Gln Gly Pro Pro Pro Pro Pro Gly
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Lys Pro Gln Gly Pro Pro Pro Gln Gly Gly Arg Pro Gln Gly Pro Pro
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Leu Leu Ser Val Ala
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Leu Ser Val Ala Leu
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Leu Ala Phe Ser Ser
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Phe Ser Ser Ala Gln
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Ala Gln Asp Leu Asp
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Leu Asp Glu Asp Val
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Gly Pro Pro Pro Pro Gly Lys Pro Gln Gly Pro Pro Pro Gln Gly
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Pro Pro Gln Gly Asp Lys Ser Arg Ser Pro Arg Ser Pro Pro Gly Lys
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Pro	Gln 130	Gly	Pro	Pro	Pro	Pro 135	Gly	ГÀз	Pro	Gln	Gly 140	Pro	Pro	Pro	Gln
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Pro	Pro	Pro	Gln	Gly 165	Gly	Asn	Gln	Pro	Gln 170	Gly	Pro	Pro	Pro	Pro 175	Pro
Gly	Lys	Pro	Gln 180	Gly	Pro	Pro	Pro	Gln 185	Gly	Gly	Asn	ГÀз	Pro 190	Gln	Gly
Pro	Pro	Pro 195	Pro	Gly	ГÀа	Pro	Gln 200	Gly	Pro	Pro	Pro	Gln 205	Gly	Asp	Lys
Ser	Gln 210	Ser	Pro	Arg	Ser	Pro 215	Pro	Gly	Lys	Pro	Gln 220	Gly	Pro	Pro	Pro
Gln 225	Gly	Gly	Asn	Gln	Pro 230		Gly	Pro	Pro	Pro 235	Pro	Pro	Gly	Lys	Pro 240
Gln	Gly	Pro	Pro	Gln 245	Gln	Gly	Gly	Asn	Arg 250	Pro	Gln	Gly	Pro	Pro 255	Pro
Pro	Gly	ГЛа	Pro 260	Gln	Gly	Pro	Pro	Pro 265	Gln	Gly	Asp	ГÀа	Ser 270	Arg	Ser
Pro	Gln	Ser 275	Pro	Pro	Gly	Lys	Pro 280	Gln	Gly	Pro	Pro	Pro 285	Gln	Gly	Gly
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Pro 305	Pro	Gln	Gly	Gly	Asn 310	Lys	Pro	Gln	Gly	Pro 315	Pro	Pro	Pro	Gly	Lys 320
Pro	Gln	Gly	Pro	Pro 325	Ala	Gln	Gly	Gly	Ser 330	Lys	Ser	Gln	Ser	Ala 335	Arg
Ala	Pro	Pro	Gly 340	Lys	Pro	Gln	Gly	Pro 345	Pro	Gln	Gln	Glu	Gly 350	Asn	Asn
Pro	Gln	Gly 355	Pro	Pro	Pro	Pro	Ala 360	Gly	Gly	Asn	Pro	Gln 365	Gln	Pro	Gln
Ala	Pro 370	Pro	Ala	Gly	Gln	Pro 375	Gln	Gly	Pro	Pro	Arg 380	Pro	Pro	Gln	Gly
Gly 385	Arg	Pro	Ser	Arg	Pro 390	Pro	Gln								

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What is claimed is:

- 1. A method of guiding a human subject's sleep schedule, comprising:
 - a) measuring the concentration of a peptide selected from the group consisting of: 1) a peptide comprising the 55 amino acid sequence PPGKPQGPPPQGGNQPQGPP-PPPGKPQ (SEQ ID NO:1), 2) a peptide comprising the amino acid sequence GNPQGPSPQGGNKPQGPPPP-PGKPQ (SEQ ID NO:2), 3) a peptide comprising the amino acid sequence SPPGKPQGPPQQEGNKPQGP-PPPGKPQ (SEQ ID NO:3), and 4) any combination thereof, in a saliva sample taken from the subject;
 - b) calculating the ratio of the concentration of the peptide(s) measured in (a) to the total amount of protein 65 in the sample, according to the equation: ([PPGKPQG-PPPQGGNQPQGPPPPPGKPQ SEQ ID NO:1]+[GN-

- PQGPSPQGGNKPQGPPPPPGKPQ SEQ ID NO: 2]+ [SPPGKPQGPPQQEGNKPQGPPPPGKPQ SEQ ID NO;3])/total protein (µg);
- c) having the subject initiate or resume a sleep schedule;
- d) measuring the concentration of a peptide selected from the group consisting of: 1) a peptide comprising the amino acid sequence PPGKPQGPPPQGGNQPQGPP-PPPGKPQ (SEQ ID NO:1), 2) a peptide comprising the amino acid sequence GNPQGPSPQGGNKPQGPPPPPGKPQ (SEQ ID NO:2), 3) a peptide comprising the amino acid sequence SPPGKPQGPPQQEGNKPQGPPPPGKPQ (SEQ ID NO:3), and 4) any combination thereof, in a saliva sample taken from the subject at one or more time points after (c), wherein the peptides of (d) are the same as the peptides of (a);
- e) calculating the ratio of the concentration of the peptide(s) measured in (d) to the total amount of protein

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in the sample, according to the equation: ([PPGKPQG-PPPQGGNQPQGPPPPGKPQ SEQ ID NO:1]+[GN-PQGPSPQGGNKPQGPPPPGKPQ SEQ ID NO: 2]+ [SPPGKPQGPPQQEGNKPQGPPPPGKPQ SEQ ID NO;3])/total protein (µg); and

f) guiding the subject's sleep schedule by modifying the duration of subsequent sleep periods using the subject's ratio(s) as calculated in (e), such that an increase in the ratio relative to the previous ratio leads to a subsequent increase in the duration of the subject's sleep period, and 10 a decrease in the ratio or a constant ratio relative to the previous ratio leads to no change in the duration of the subject's sleep period or a subsequent decrease in the duration of the subject's sleep period.

* * *